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EDITED BY

SIR RAY LANKESTER, K.C.B., M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE,
AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY
OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES
OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF
GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND
OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN
ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE
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OF THE ROYAL ZOOLOGICAL AND MALACOLOGICAL SOCIETY OF BELGIUM;
CORRESPONDING MEMBER OF THE SEYDITZBERG ACADEMY OF FRANKFURT-A-M;
FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE
AMERICAN PHILOSOPHICAL SOCIETY;

HONORARY FELLOW OF THE ROYAL SOCIETY OF EDINBURGH;
LATE DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE PRESIDENT OF THE
BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE; LATE FULLERIAN PROFESSOR OF
PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN.
LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD;
EMERITUS PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN UNIVERSITY COLLEGE, UNIVERSITY OF LONDON.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN THE UNIVERSITY OF CAMBRIDGE

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF MANCHESTER;

AND

E. A. MINCHIN, M.A.,

PROFESSOR OF PROTOZOOLOGY IN THE UNIVERSITY OF LONDON.

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Early Ontogenetic Phenomena in Mammals and their Bearing on our Interpretation of the Phylogeny of the Vertebrates.

By
A. A. W. Hubrecht.

With 160 Text-figures.

PREFACE.

In the present paper I have attempted to bring together the results of new investigations and recent reflexions with such as had already been published on earlier occasions, but which, having appeared in very different periodicals and publications ('89, '90, '94, '95, '96, '99, '02, '05, '07), could not be easily brought into the necessary connection with each other by the reader.

I have to thank my friend Sir Ray Lankester for giving me the occasion to present this scattered material in a more concise form, and for his willingness to admit a profuse quantity of process figures into a JOURNAL which, under his direction, has become justly famous for its excellent lithographic plates.

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CHAPTER I. THE EARLIEST CELL-LAYERS.

INTRODUCTORY.

The phenomenon of fecundation of the egg inaugurates the well-known series of cell-divisions which give rise in *Amphioxus* to a grouping of the first cleavage-cells into a hollow mulberry shape, whereas in cartilaginous fishes, in reptiles, and in birds the cleavage-cells are disposed in disc-shape at one point of the yolk, which latter, though originally part of the egg, will soon take the aspect of an appendage to the embryo. Again, in *Amphibia* and in certain more archaic fishes the yolk is much less considerably developed and the whole egg thus segmented in toto, whereas in the *Teleosts* there is an abundance of food-yolk, but a disposition of the parts somewhat different from what we find in cartilaginous fishes and in *Sauropsids*.

In *Mammals* again the whole of the egg-substance is segmented (holoblastic cleavage as against the meroblastic cleavage of the cartilaginous fishes and the *Sauropsida*), but the further development more and more resembles that of the reptiles in which a very considerable yolk is present, a fact that has given rise to the erroneous conclusion that the mammalian blastocyst was derived from the *Sauropsidan* by a process consisting in the gradual disappearance of the yolk, with retention of the other developmental characters. We will find occasion later on to discuss the value of this phylogenetic speculation.

We will in this chapter have to consider the earliest processes by which the cell-material consequent upon the cleavage of the egg comes to be arranged in the fundamental cell-layers out of which the different organs of the adult animal will gradually take their origin.

And we must in the first place call attention to numerous and important investigations that have taken place, more particularly concerning invertebrate animals, in which the cleavage-cells were followed as far as possible up to their final destination with respect to organogenesis (Wilson, '92, '97; Conklin, '97; Casteel, '04).

These researches concerning the "cell-lineage," as it has been called, have been carried on by the aid of such worms and molluscs that had eggs as transparent as possible, and, notwithstanding the evident high importance of the results obtained, there is for the present little chance of success for similar attempts with the opaque and yolk-laden or deeply hidden eggs of the Vertebrates. I mention this in order to point out that several questions in dispute might in this way be settled, and that more especially the mammalian egg with its holoblastic cleavage would here offer a most desirable object of study. There has been a tendency to suppose that the two primary cell-layers which are encountered in all vertebrate and invertebrate animals, the ectoderm and the endoderm, already become separated from each other when the two first cleavage cells arise.

Others have concluded that in this separation of the egg-cell into the two first cleavage cells the embryonic material was separated into the mother-cells of the right and the left half of the body or into the anterior and posterior half, as chance would have it (Roux). Experiments have even been carried out to prove this. At the present moment we are not in a position to say whether there is any general rule in this respect applicable to all vertebrates, and yet there seems to be hardly any doubt that both in *Amphioxus* and in man—the two opposite extremes in the phylum of the Chordata—the two first cleavage cells, if separated from each other,

may under favourable conditions each of them develop into a perfect, full-grown individual.

However this may be, the formation of the primitive cell-layers out of the cell-material derived from the segmenting egg-cells must now, in the first place, be considered, and, inverting the order generally followed, we will begin by considering the phenomena as they present themselves in the

A. MONODELPHIAN AND DIDELPHIAN MAMMALS.

As yet only a restricted number have been investigated with regard to the process of cleavage and the earliest formation of the layers, it being no easy matter to procure the material. As such I mention—

(1) Certain species of Primates,¹ including both monkeys (*Macacus*, *Cercopithecus*, a. o.) by Slenka ('99, '00) and by Keibel ('04), and *Tarsius* by myself ('02).

(2) Lemurs (*Nycticebus*) by myself ('07).

(3) Carnivores (dog and cat) by Bonnet ('97) and Duval ('94, '95).

(4) Chiroptera (diverse species of *Vespertili*) by E. van Beneden and Ch. Julin ('90) and by Duval ('99).

(5) Insectivora (*Talpa*, *Erinaceus*, *Gymnura*, *Sorex*, *Tupaja*) by Heape ('83), Keibel ('88), and myself ('89, '90, '95, '98).

(6) Rodentia (*Lepus*, *Mus*, *Arvicola*, *Cavia*, *Sciurus*, a. o.) by Hensen ('76), E. van Beneden ('80), Slenka ('83, '84), Fraser ('82), Masius ('89), Fleischmann ('91), Keibel ('80), Duval ('92), Robinson ('92), a. o.

(7) Ungulata (*Ovis*, *Sus*, *Cervus*) by Bonnet ('82), Keibel ('93), Assheton ('98), Weyssse ('94), a. o.

(8) Dermoptera (*Galeopithecus*) by myself.

(9) Edentata (*Manis*) by myself.

(10) Didelphia (*Opossum*, a. o.) by Slenka ('87).

¹ Of the human subject no such early stages have as yet been brought to light, the earliest being those of Peters, von Heekelom, Bryce and Teacher.

Fresh eggs have served for the observation of the cleavage-processes in the rabbit to van Beneden and in the bat to van Beneden and Julin. Most of the other authors have made use of preserved specimens and of sections. A certain number of the figures given by different observers are here reproduced (Figs. 1—36).

1. The Mammalian Morula.

The compact mulberry stage (different in its compactness from the hollow mulberry of the holoblastic egg of *Amphioxus* alluded to above) contains about 36—72 cells. In the case of *Tupaja* and—judging from Bonnet's figure—of the dog the central cell or cells show a different reaction against staining reagents than the peripheral (Figs. 1, 2, 3). We will have occasion to discuss this phenomenon later on. Very soon fluid begins to accumulate between some of the constituent cells of this mulberry stage in mammalian development, and the solid mulberry then becomes converted into a hollow sphere, against the wall of which an accumulation of cells is visible which was already noticed by Bischoff ('42, '45) and other early authors.

Twenty-five years ago, when van Beneden published his remarkable researches above alluded to on the early development of the rabbit, the interpretation of these early phenomena was far from being satisfactory or uniform. The so-called metagastrula stage of mammals, first described by van Beneden ('80), has since been abandoned by that author [though taken up again by Duval ('99, p. 64)]. We may, however, say that of late years a very general consensus of opinion has come to be established. In all the orders above-mentioned an early stage of the blastocyst has been observed corresponding to the phase just described in which an accumulation of cleavage-cells adheres at one point against an outer epithelial layer.¹

¹ E. van Beneden has ascribed the origin of the free space between the



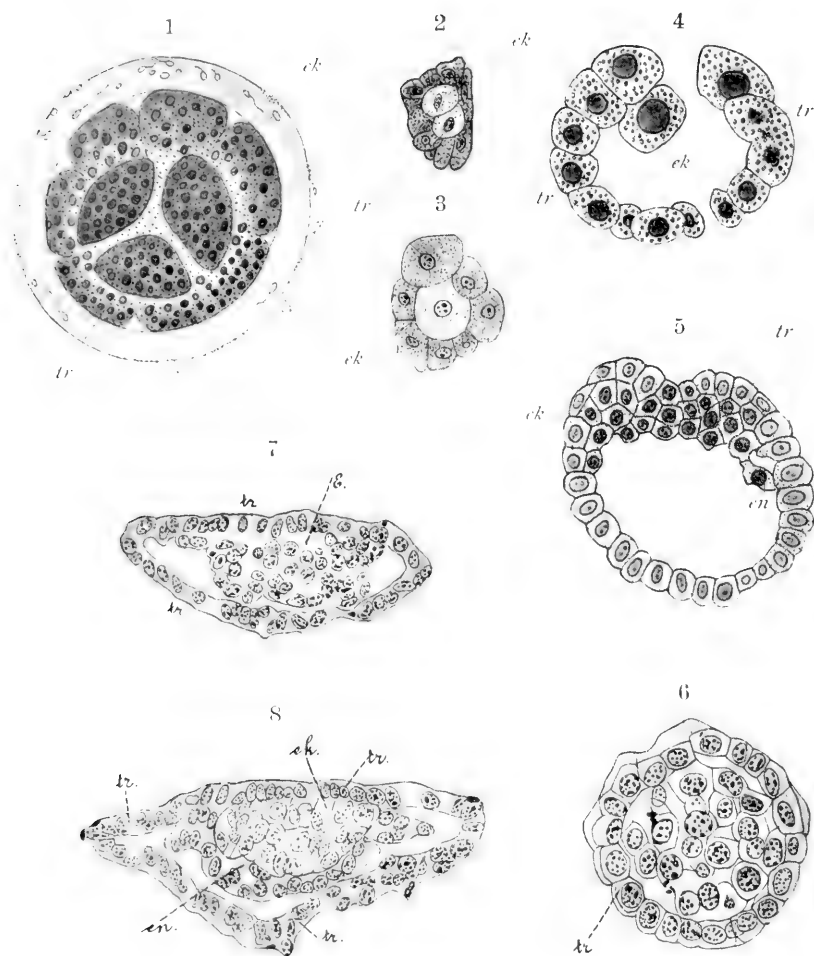


Fig. 1. Cleavage stage of the dog (after Bonnet, '97). The mother cells *ek* of the embryonic knob, centrally situated, have stained more deeply than the trophoblast cells (*tr*). — Fig. 2 and 3. Sections through two different early cleavage stages of *Tupaja javanica*. In this case the trophoblast cells, *tr* are more deeply stained than the mother-cells of the embryonic knob *ek*. — Fig. 4 and 5. Sections through early stages of the opossum (after Selenka, '87). In Fig. 4 there are thirteen trophoblast cells *tr* and one mother cell of the embryonic knob *ek*, in Fig. 5 the latter has given rise to a mass of cells which begins to project on the surface (*ek*) and in which the differentiation of entoderm cells has just commenced. — Fig. 6, 7, 8. Three sections of different developmental stages of the bat (after van Beneden, '99). In Fig. 6 the differentiation between trophoblast cells *tr* and embryonic knob is again expressed in the staining; in Fig. 7 the embryonic knob (*E*) is not yet separated into ectoderm (*ek*) and entoderm (*en*) as it is in Fig. 8.

This outer layer has been termed by me the trophoblast, that inner cell-mass: the embryonic knob or "Embryonalknoten" ('88, p. 511; '89, p. 298). E. van Beneden, while recognising that the trophoblast is a separate layer ('99), does not as yet apply that name but calls it somewhat more circumlocutionally the "couche enveloppante."

The degree of independence existing between trophoblast and embryonic knob is subject to considerable variation. As the two only become segregated when the change of the morula stage into a vesicle comes about, there is generally at the outset no such very sharp distinction, and even in later stages this distinction is sharper in certain genera of mammals (Tupaja, Figs. 21, 29; Galeopithecus, Fig. 18; Cervus, Figs. 13, 14) than in others (Erinaceus, Figs. 33—36; Tarsius, Fig. 19; Cavia, Fig. 24. Still there is reason to believe that in some it may be traced up to the very early stages of cleavage as are indicated by Figs. 1—3. The embryonic knob would then be represented by one or a few central cells, the trophoblast by the surrounding cleavage cells (as was already noticed above).

When the trophoblast is being distended into a vesicle the proliferation of the mother-cells of the embryonic knob is very much slower; the total number of cells of which the knob is built up rarely exceeding 24—30.

For the Opossum we have the data furnished by Selenka ('87), to which, however, I would put another interpretation. The central cell of Fig. 4, looked upon by him, without further proof, as a hypoblast cell, is undoubtedly the mother-cell of the embryonic knob as a comparison with Fig. 5 makes all the more evident. It is, of course, important to find this agreement between didelphic and monodelphic mammals.

epithelial outer layer and the inner mass to the extension of intracellular vacuoles ('99). His interpretation has found no support in the results obtained by Keibel and myself, nor in those of Selenka for the Opossum.

2. The Origin of the Entoderm.

Soon a most important process is inaugurated and from the inner cell-mass arises by delamination a separate lower layer which we designate as the entoderm of the embryo. These entoderm cells wander in radial direction along the inner surface of the trophoblast, which, in many cases, is thus soon transformed into a didermic structure. Sometimes, as for instance in *Tarsius* (Hubrecht, '02) the more important part of the delaminating entoderm (viz. that which remains situated below the rest of the embryonic-knob-cells) is present as a distinct cell-layer before this migration of entoderm cells towards the inner surface of the trophoblast commences (Figs. 8, 19); in other cases (*Sorex*, *Lepus*, a. o.) the entoderm cells migrate as soon as formed; whereas in *Tupaja* it is only after the entodermic vesicle has become nearly completed that the part of it which will remain in contact with the embryonic ectoderm is separated off from the latter by delamination (Fig. 29).

In certain mammals (*Tarsius*, monkeys, man) the entoderm cells never clothe the whole of the inner surface of the trophoblast, the entodermic vesicle remaining of smaller size than the trophoblastic sphere (Figs. 39, 40, 44—46, 62—65). To a certain extent this is explained by the fact that another vesicle (of which mention will be made later on) develops, at an uncommonly early period, fills up part of the vesicle formed by the trophoblast and prevents the entoderm cells from reaching the entire outer surface.¹

When the entoderm has separated off by delamination from

¹ It would seem that in *Erinaceus* a similar state of thing occurs temporarily, it having been observed by me ('89, Figs. 25, 26) that a closed entodermic vesicle, far smaller than the trophoblastic sphere which encloses it, is here noticed in very early stages (Fig. 34). Shortly after this (Figs. 35—38) the hedge-hog's blastocyst, is, however, a spherical trophoblast, against which the endoderm is everywhere adherent. The investigation of numerous early stages of the hedge-hog is, however, yet necessary to settle this point.

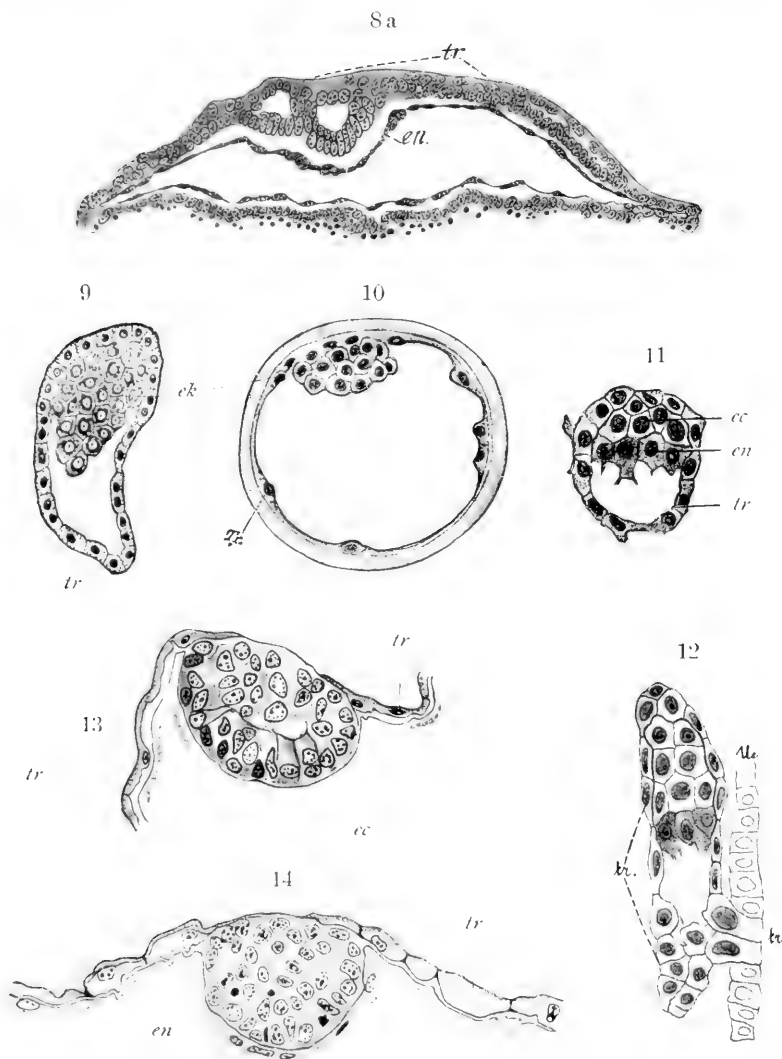


Fig. 8a. Section through an early bat's blastocyst (cf. Fig. 6 to 8); *tr* trophoblast; *en* entoderm. The ectodermic shield has not yet emerged out of its trophoblastic covering (after van Beneden, '99). — Fig. 9. Section through early stage of the bat (after Duval) *ek* embryonic knob. *tr* trophoblast. — Fig. 10. Section through early stage of the shrew (after Hübner, '90). *ek* embryonic knob, *tr* trophoblast. — Fig. 11 and 12. Sections through early stages of the mouse, before and after fixation of the blastocyst to the uterine epithelium *u*. — Fig. 13 and 14. Two sections through the embryonic knob of *Cervus* (after Keibel, '99) before and after the development of the entoderm by delamination. The trophoblast (*tr*) is quite distinct from the embryonic ectoderm *ec*; in Fig. 13 ectoderm and entoderm are yet united in the embryonic knob.

the embryonic knob, the remaining cells of the latter form the "embryonic ectoderm," which is thus situated between the entoderm and the trophoblast, and could for that reason easily deceive van Beneden ('80) in regarding it as a third, mesodermic layer (Figs. 8, 11, 12, 15, 17, 18, 21—23).

3. Developmental phases of the Didermic Embryonic Shield.

The portion of the mammalian blastocyst where the embryonic ectoderm and its subjacent entodermal layer are situated may, already at this early stage, be conveniently termed the embryonic shield. This shield is sometimes slightly convex with the ectoderm on the convex side (rabbit, Fig. 23), sometimes it is bent the other way (Sus, Fig. 17; Tupaja, Figs. 29—31; Tarsius, Figs. 20, 45), sometimes first one way (Figs. 20, 37, 53), and later (Figs. 50, 38) the other (Tarsius, hedgehog). Sometimes it is (Fig. 23) quite flat (Lepus, Sorex, a. o.).

A very instructive and in my opinion very archaic case among those above-mentioned is that in which the embryonic shield remains separated from the overlaying trophoblast by a space which arises simultaneously with the growing blastocyst. This space is from the outset a lenticular or crescentic cavity. Its appearance in *Erinaceus* is elucidated by the accompanying diagrams (Figs. 36—38). It represents the most typical instance of the manner in which the earliest amnion may have arisen as a protective water-cushion between the trophoblast and the embryonic shield, and we shall later on see that the space within the hedgehog's amnion is actually a later development of this early cavity. In the bat slight modifications of this simple arrangement occur, which seem to lead on to arrangements as we find them in *Tarsius* and in many Ungulates and Rodents, whereas on the other hand *Pteropus* (Figs. 22, 72), *Galeopithecus* (Figs. 41, 42), *Cavia* (Figs. 24, 25), monkeys and man (Figs. 39, 40) have developed

along another path, along which the amniotic cavity has from the first remained a closed vesicle.

A very important case is that of *Tupaja* in which the embryonic shield is originally quite bent upon itself (Fig. 30), convexity inwards, and gradually expands (under rupture and dehiscence of the trophoblast) into a flat surface with no trophoblastic covering above it (Fig. 32) by successive stages as they are reproduced in the accompanying diagrams. This arrangement possesses suggestive points of comparison with what has been called by Selenka ('00 *a*, p. 201) the "Entypie" of the embryonic shield, such as it exists in many rodents. All these cases are variations upon a similar theme as that of *Tupaja*, and not necessarily (as Selenka would have it) a consequence of the blastocyst undergoing its development in a cavity of exiguous dimensions, to the walls of which it had early adhered. *Tupaja* at once does away with this argument (Hubr., '99 *B*, p. 173), because here the blastocyst, while free from any attachment to the uterine walls, has yet the appearance of Figs. 30 and 31. The causes of the folded condition of the embryonic shield can hardly be so simply mechanical as Selenka supposed. They remain obscure for the present and will come anew under consideration when the origin of the amnion will be discussed.

The point to which the facts here brought forward have led us is the recognition that during the development of the mammalian blastocyst the trophoblast, which originally encloses the embryonic knob, behaves very differently in various mammals at the period that out of this knob arises the embryonic shield with its didermic arrangement of the cells out of which the embryo is going to be built up. In the hedgehog (Figs. 37, 38), in *Gymnura*, in *Pteropus* (Figs. 67, 68), and in the other bats hitherto examined (Fig. 8*a*), in *Galeopithecus* (Figs. 41, 42), in many rodents (*Arvicola*, *Mus*, *Cavia*, Figs. 24—28), in monkeys, and (most probably) in man the trophoblast remains an entirely closed vesicle, inside of which the ontogenetic development of the embryonic knob will follow its course. In other genera of

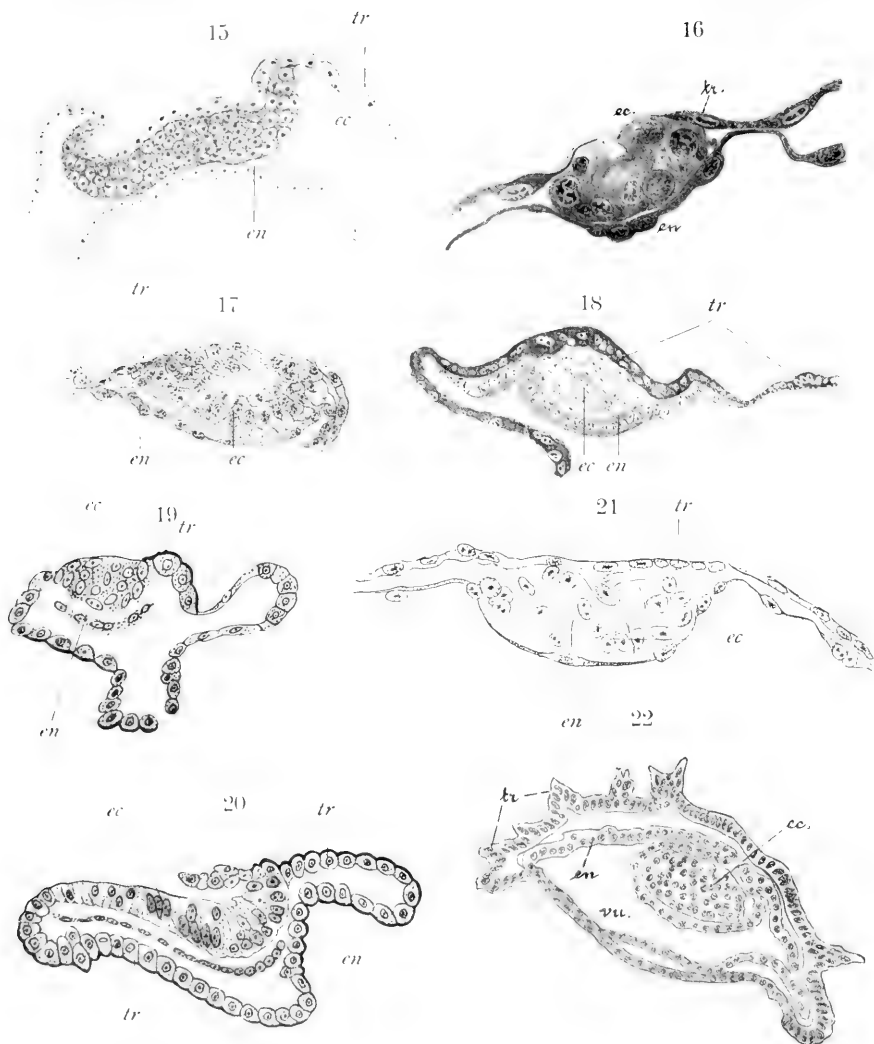


Fig. 15. Section through a similar stage of *Amnospermophilus*. After a not yet published drawing of Prof. G. Lee of Minneapolis. Trophoblast *tr* continued over embryonic ectoderm *cc*, *en* entoderm. — Fig. 16. The same for the sheep (after Assheton, '98). — Fig. 17. The same for the pig (after Weyss, '94). The ectoderm has not yet opened out on the surface of the blastocyst (cf. Fig. 29–32). — Fig. 18. The same for *Galeopithecus*. — Fig. 19 and 20. The same for *Tarsius*. In Fig. 19 the entoderm *en* is in the very earliest phase of delamination (after Hubrecht, '02). In Fig. 20 there are yet remnants of the trophoblastic covering of the ectodermic shield. — Fig. 21. The same for *Tupaja* (after Hubrecht, '95). — Fig. 22. The same for *Pteropus*. In the ectodermal knob (*ec*) a cavity will soon appear which becomes the amnion cavity (after Selenka and Göhre, '92). *vu* umbilical vesicle.

mammals that part of the embryonic knob which is going to be the ectoderm of the embryonic shield rises to the surface, interpolates itself between the trophoblast cells, which then form no longer a closed sphere, but one that is discontinuous by the fact that at one pole this ectodermic shield has replaced what were originally trophoblast cells. This displacement may come about as it does in *Tupaja* (Figs. 29—32) where the unfolding of the embryonic shield bursts open the trophoblastic covering above the shield, thus increasing the surface of the vesicle by an area which is not trophoblast, but embryonic ectoderm. Or it may happen that a similar but less distinct process of dehiscence interpolates embryonic ectoderm in the trophoblastic vesicle in the way it comes about in the *Opossum* (Fig. 5), *Tarsius* (Fig. 20), *Cervus* (Fig. 13), *Sus* (Fig. 17), *Ovis* (Fig. 16). Or finally the trophoblast may continue to cover the embryonic ectoderm as in the case first named, but without the development of any cavity between it and the embryonic shield (Fig. 15). In this latter case, of which the classical example is the rabbit, as it was so clearly figured by Kölliker (Fig. 23), the trophoblast cells covering the embryonic ectoderm flatten out considerably, and finally disappear. Another example of this is the shrew (Hubrecht, '90; Fig. 26). These flat cells—superposed to the embryonic ectoderm—were for a long time designated as Rauber's cells, Rauber having been the first to direct attention to them. It was, however, not observed by Rauber, as it was later so clearly noticed by Kölliker, that this layer is merely the continuation of the peripheral trophoblast cells, but it remained for a long time an accepted, though erroneous interpretation, that the embryonic ectoderm was uninterruptedly continued in the peripheral trophoblast, and that Rauber's cells were an additional arrangement. This error was a natural consequence of a comparison, on a false basis, hereafter to be corrected, of the mammalian with the avian and reptilian blastocyst. The opinion of certain authors (Balfour,

Heape) that some of the Rauber cells become incorporated into the embryonic shield has not been well established nor been confirmed of late. I incline to believe in their final disappearance, and wish to call attention to the transition case which we may notice, for example, in *Tarsius* (Hubrecht, '02; Figs. 49*a*, *b*, 50*b*), where trophoblast cells can remain for yet a considerable time attached to the embryonic ectoderm, but also finally disappear. In this case the trophoblast opens up according to the type prevalent in the second group described above, and the permanence of an isolated trophoblast cell on the embryonic ectoderm is a matter of chance.

We may, in concluding this exposition of the varied relations in which trophoblast and embryonic ectoderm stand to each other in mammals, insist upon the fact that—if we except the *Ornithodelphia*, which will be discussed hereafter, and are as yet barely known as far as their early ontogeny is concerned¹ (Caldwell, '87; Semon, '94; Wilson and Hill, '03)—all the *Didelphia* and *Monodelphia* hitherto investigated show at a very early moment the didermic stage out of which the embryo will be built up enclosed in a cellular vesicle (the trophoblast), of which no particle ever enters into the embryonic organisation.

4. The Mammalian Gastrula.

The didermic stage of the mammalian blastocyst just alluded to fully deserves the name of the "gastrula" stage as I have elsewhere attempted to expound ('02, p. 65—75; '05, p. 408). We should bear in mind, as was noticed in the introduction, that comparative ontogeny has come to a deadlock when attempting to fit in the mammals into the current interpretation of the early development of vertebrates. To

¹ Just lately the more extensive paper of Wilson and Hill ('07) has appeared, in which figures are given (pl. 2, figs. 4, 5), which allow us to accept quite similar arrangements for the *Ornithodelphia* (see text-figs. 66—70).

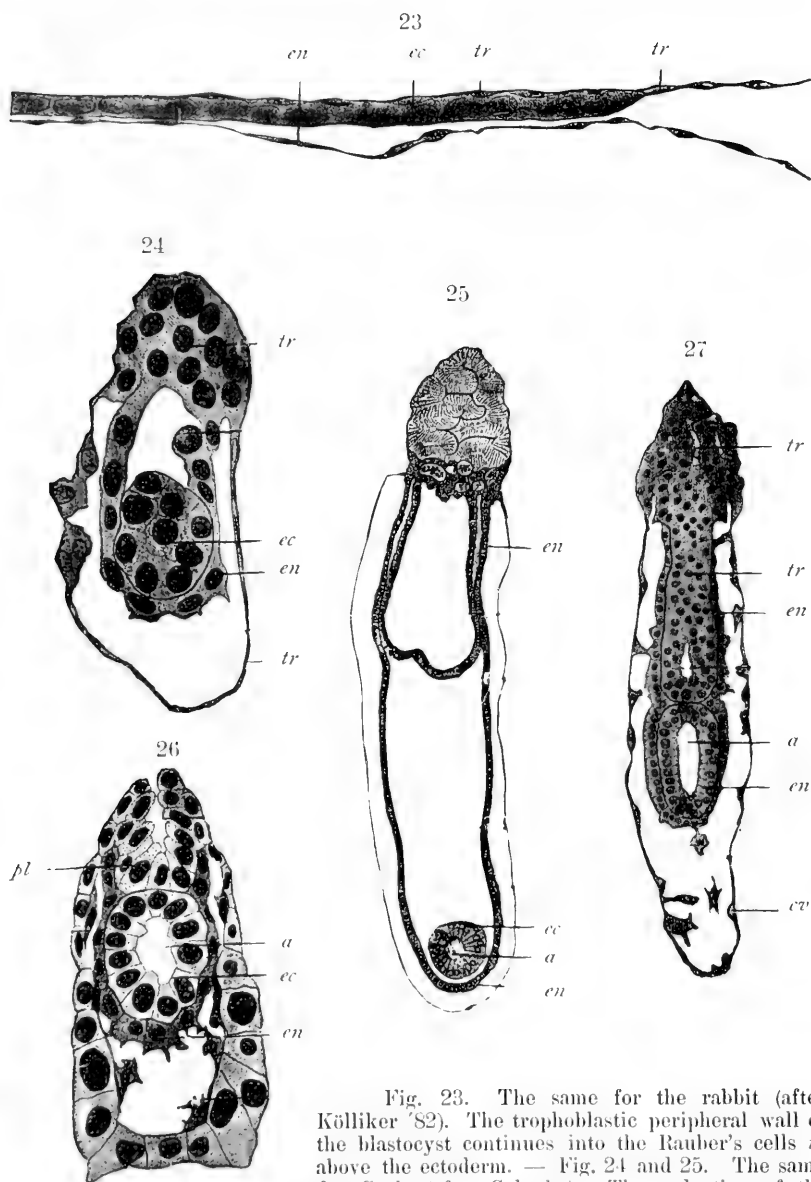


Fig. 23. The same for the rabbit (after Kölliker '82). The trophoblastic peripheral wall of the blastocyst continues into the Rauber's cells *tr* above the ectoderm. — Fig. 24 and 25. The same for *Cavia* (after Selenka). The reduction of the trophoblast is yet far more considerable. *a* amnion cavity. — Fig. 26 and 27. Sections through two early stages of the mouse's blastocyst (after Selenka, '83). The trophoblast (*cv*) is much further reduced in the second than in the first whereas that part of it (*pl*) which will form the placenta has proliferated much more considerably. *a* amnion cavity.

express it in O. Hertwig's own words: "Die grössten Schwierigkeiten bereitet den Embryologen die Keimblattbildung bei den Säugethieren . . . wegen der von anderen Wirbelthieren stark abweichenden Befunde" ('06, p. 898).

As soon as we separate the phenomena of notogenesis, such as they are found in all vertebrates—*Amphioxus* included—from the phenomenon of gastrulation, recognising that the former follow upon the latter and bring about the formation of the notochord and the mesoblastic somites, the difficulties are considerably simplified.

Gastrulation is thus terminated in the mammalia when the didermic stage of the embryonic shield has come into existence. We have seen that this takes place not in consequence of any process of invagination but by means of a most unmistakable delamination of the entoderm, out of the embryonic knob.

This delamination gastrula of the mammalia generally enters upon the later phases of ontogeny which will be described hereafter without the appearance of a distinct blastopore.

Still to this there are a few notable exceptions that have gradually come to light, but have been mostly overlooked or misinterpreted in consequence of the erroneous views above alluded to. The most striking example is undoubtedly offered by the hedgehog, where the blastopore, a clearly visible perforation towards the hinder end of the embryonic shield, makes an evanescent appearance at one particular stage of the individual development (Fig. 53).

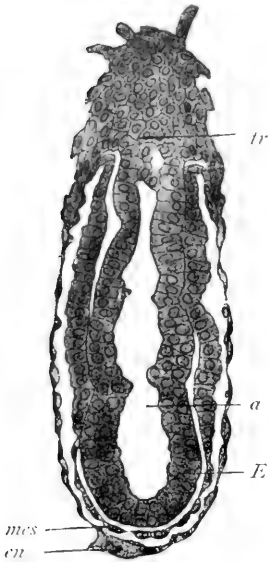
Along the lips of this opening the ectoderm and entoderm pass into each other, whereas these two layers, although genetically related, have up to this moment been separated and nowhere in confluence with each other. This latter fact is recognised by all observers. I am inclined to believe that the formation of the blastopore in the hedgehog is not only very evanescent, but that it does not necessarily appear in all hedgehog-embryos, and that in exceptional cases the formation of notochord and somites may commence without the blastopore having become a visible opening.

In *Tarsius* on the other hand, where in an overwhelming number of cases the embryonic shield undergoes the changes consequent upon the first appearance of notochord and somites without any faint trace of a blastopore, one quite exceptional case came under observation (Fig. 52) in which what was evidently an atavistic attempt in that direction was noticed; all the more important because it helps us to fix the spot in the didermic gastrula at which the blastopore naturally occurs. Similarly blastoporic openings, or attempts at such a perforation in these early stages, have been noticed in the rabbit by Keibel ('89; Figs. 46, 47), in the mole by Heape (Fig. 54), in the opossum by Selenka (Fig. 55), in the shrew by myself (Figs. 56 and 57). In the diagrams a few of these observations have been reproduced.

The gastrula stage and the blastopore of the mammalia are thus limited to the early phases and the simple phenomena here described. The blastopore becomes closed in all the cases above noticed, and after that a series of processes are initiated in which it would be utterly misleading further to use the word blastopore, *Gastrulamund*, *Urmund*, or *Urmundlippen*. These structures in the further development that have been thus termed ought to be termed differently if we wish to put an end to the confusion that obscures these points at the present moment.

At the same time it should be noticed that one of the first features by which the formation of the notochord begins, viz., the formation on the embryonic shield of that median ectodermal proliferation, which I have called ('90) the protochordal wedge (*Primitivknoten*, Bonnet = Hensen'scher *Knoten*), takes place in the identical spot where the evanescent blastopore was or is situated (Fig. 52); and that from this point backwards a median region of proliferation extends which on O. Hertwig's example has been called the homologue of the "*Urmund*" and the "*Urmundlippen*," but which we ought to compare as I have elsewhere advocated ('02, '05) with an elongated stomodæal slit, which even in the hypothetical ancestral forms was no longer a blastopore, but

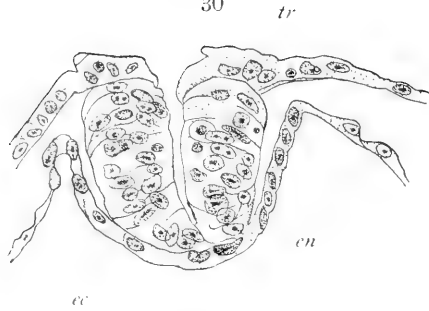
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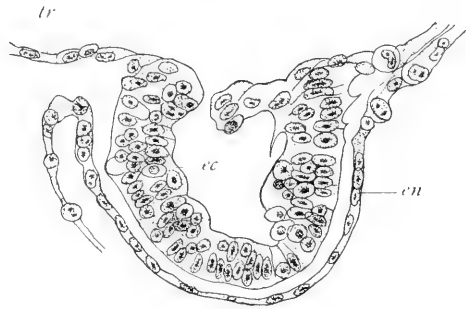
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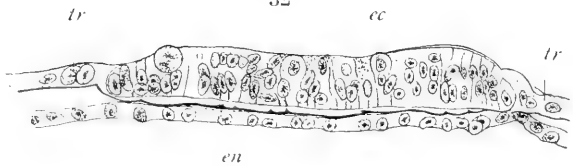


Fig. 28. Section through an early blastocyst of the mouse (after Selenka, '83). *a* amnion, on the point of being constricted off. *E* Ectodermal shield. *mes* mesoblast. *en* entoderm. *tr* trophoblastic rudiment of placenta. — Fig. 29 to 32. Four successive stages in the early development of *Tupaja javanica*. In Fig. 29 the trophoblast *tr* yet forms a solid closed sac round embryonic knob and entoderm, the latter only just beginning to split off from the embryonic knob as far as its embryonic portion is concerned. In Fig. 30 and 31 the bent embryonic ectoderm *ec* commences to free itself from its trophoblastic covering; in Fig. 32 it has quite flattened out, forming the embryonic shield on the top of the spherical blastocyst.

a dorsal mouthslit, a "Rückenmund" (Fig. 160) of a vermactinial stage of development.

The mammalian blastopore, rudimentary, rare, and evanescent as it is, still reminds us of the blastopore of the invertebrates in this respect that in its immediate vicinity those cell proliferations commence which lead up to the formation of the so-called mesodermal structures.

5. Theoretical Speculations about the Origin of the Trophoblast.

The facts with which we have up to now become acquainted concerning the early development of didelphic and monodelphic mammals (the so-called marsupials and the placental mammalia) fully justify the conclusion that the embryo already in its very earliest ontogenetic phases is provided with a larval envelope, an "Embryonalhülle." To this layer of cells we have given the name of trophoblast. Later on we shall see that this layer, though it is at the outset only one cell thick, can undergo the most varied proliferations in very divergent spots, and that such proliferations are at the basis of the whole phenomenon of placentation. The fact that to these proliferations and their significance for the early nutrition of the embryo, attention was first directed (Hubrecht, '88, '89) before the more general significance of the layer as a larval envelope had yet been fully appreciated was the cause that the name of trophoblast has been given to it.¹ We will return to this when the phenomena of placentation will be discussed.

It cannot be denied that the consequences of considering the trophoblast as a larval envelope and of introducing this

¹ It remains to be seen whether the name of "trophoderm," introduced by Sedgwick Minot ('03) for that portion of the trophoblast which takes an active part in placentation, is a desirable innovation, or rather a synonymous encumbrance. But even in the former case Duval's proposal of the name of "ecto-placenta" has the priority.

generalisation into the developmental history of vertebrates may be far-reaching.

Up to now foetal envelopes or membranes were only known in the ontogeny of reptiles, birds and mammals at somewhat later stages of their development. These membranes were respectively known as amnion, chorion, serous membrane, subzonal membrane (and in case of the Sauropsids and certain mammals, even allantois) so that Milne Edwards' subdivision of the vertebrates into Amniota, Allantoidea, as against the Anamnia, Anallantoidea, was based on the presence or absence of such membranes. Of the phylogenetic evolution of these foetal membranes no reasonable explanation has yet been offered, as is, for example, recognised, as far as the amnion is concerned, in an unbiassed handbook of human embryology, as is that of Sedgwick Minot (p. 344, 1st edition). Now this obscure phylogeny would seem to become yet more complicated when we add to the already existing foetal membranes a new larval envelope, called trophoblast. The case is, however, quite the contrary. This early envelope, that we have seen making its appearance soon after the very first phases of segmentation of the mammalian ovum, instead of adding new difficulties, helps to explain old ones. It throws new and unerring light on the first origin both of the amnion and the chorion (respectively: serous membrane) and may prove to be a valuable key that may lead to a reasonable interpretation of much that is as yet obscure and incomprehensible. Out of this very earliest larval envelope the others seem to have gradually evolved; they may be looked upon as further differentiations of it and we have now to look out for the first origin of the trophoblast itself and see if we can furnish a hypothesis worthy of further consideration. In that case the phylogeny of the other foetal membranes would *à fortiori* have been explained at the same time.

Now, I believe that we have only to assume that the ancestors of those Vertebrates in which a distinct trophoblast or the traces of it are found, were already possessed of a larval envelope in the antecedent stages of phylogeny, in order to

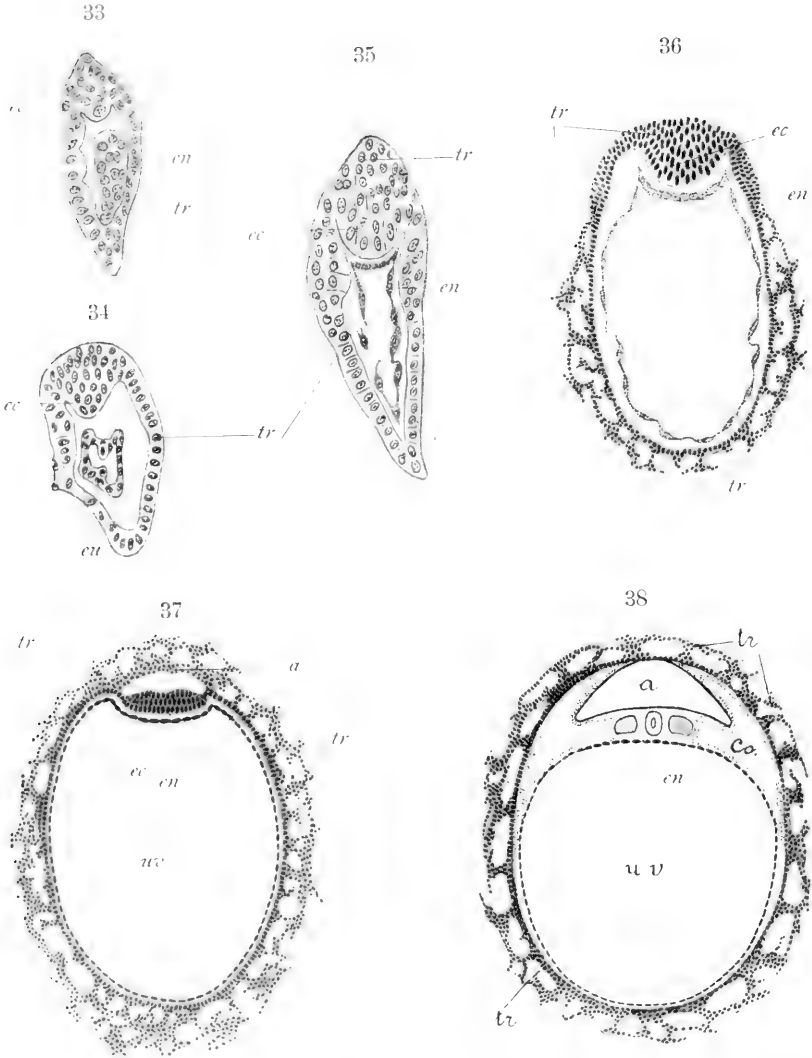
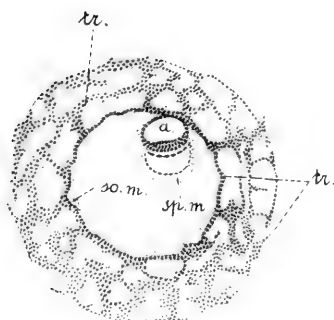
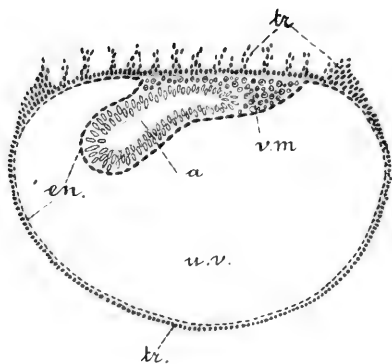


Fig. 33, 34 and 35. Sections of quite early stages of the hedgehog's blastocyst. *Tr* trophoblast, *en* entoderm, *ec* ectoderm yet firmly united with the trophoblast. Fig. 36. A somewhat later stage in which considerable lacunae have originated in the proliferating trophoblast into which maternal blood penetrates. Fig. 37. Section through a yet later stage in which the lacunae have developed all round the blastocyst and in which the amnion cavity (*a*) has arisen as a split between trophoblast and embryonic ectoderm (*ec*). — Fig. 38. Yet later stage of the hedgehog's blastocyst in which the development of the embryo is further advanced and the amnion-wall completed and externally clothed by mesoblast. *uv* umbilical vesicle, *co* coelom.

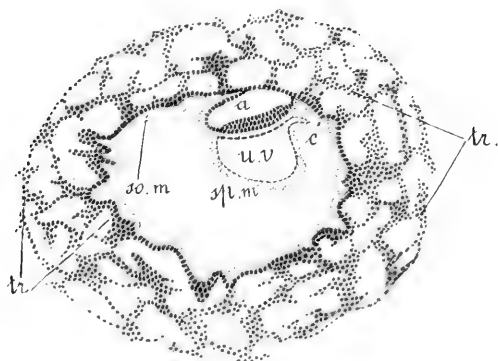
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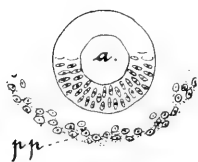
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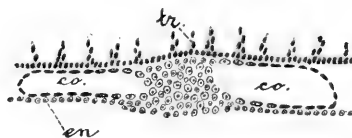


Fig. 39 and 40. Diagrammatic sections through two stages in the early blastocyst of man and the anthropomorphae, combined out of Selenka's ('99, '00) and Peters' (99b) drawings. *c* connective stalk, *som* & *spm* somatic and splanchnic mesoblast. Amnion and trophoblast as in the hedgehog. — Fig. 41 and 42. A longitudinal and a transverse section of an early developmental stage of *Galeopithecus volans*. In Fig. 41 the placenta is commencing to be formed on the upper surface of the blastocyst. Here too the amnion cavity (*a*) has arisen by dehiscence inside the embryonic knob. *vm* ventral mesoblast, connecting embryonic region with trophoblast. Fig. 42 belongs to a somewhat later stage in which a thickening in the entoderm (protochordal plate) has become visible. — Fig. 43. Transverse section through the ventral mesoblast of *Galeopithecus*. *co* coelom, *en* entoderm, *tr* trophoblast.

obtain such a working hypothesis. Both Sauropsids and Mammalia are, *omnium consensu*, phylogenetically derived from very early Protetrapods that lived about the Carboniferous period or even earlier, and which, in their turn, had aquatic and fish-like progenitors. These early, to us unknown, fishes have sprung from vermiform predecessors of coelenterate pedigree.

A tendency to exchange the radial for a bilateral symmetry and to separate the coelom from the enteron must at one time have characterised certain coelenterate ancestral forms, as has already been advocated by Sedgwick ('84) and by myself ('05) on earlier occasions. It is not straining the imagination to assume that in this line of descent closely-related forms may have developed, some with, others without a larval envelope, temporarily ensheathing the cellular elements that will build up the embryo itself and thus foreshadowing the separation among their later, vertebrate descendants of such with and such others without a trophoblast.

We find examples of this amongst the Nemertean worms. In some of these the egg after segmentation develops straight away into the young worm, in others, which as far as the typical Nemertean characteristics go are very closely related, the cleavage results in a disposition of the embryonic material into (*a*) the first lineaments of the embryo itself and (*b*) a cellular temporary envelope of these, which is either more closely applied to (Desor's larva) or more distant from (Pilidium larva) the material that goes to build up the embryo.

And though I in no way want to infer that it is among the Nemertea or Gephyrea that we would have to look for the ancestral forms of the Vertebrates (nor either amongst any of the Annelids known to us) still it is an instructive fact that among different classes of worms (Gephyrea should also here be mentioned, see Fig. 129) the larval envelopes above alluded to are encountered in some but are absent in others.

This particularity may have passed on in the ancestral line of the chordata.

Now, if in our further phylogenetic speculations concerning the Protetrapods and their descendants that live at the present time, we were to start from an oviparous aquatic animal, whose early developmental stages are provided with a larval envelope, we understand that, when any such animal came to adapt itself to inhabit the dry land it would doubtlessly score certain advantages, if at the same time it became viviparous. Its adaptation would certainly be the more complete if, for its reproduction, it were independent of the aquatic medium. And towards the efficiency of this viviparous condition the larval envelope could immediately contribute by the mere change of its protective or locomotor significance into an adhesive one. This again would be facilitated if the larval envelope, increasing in surface, were to develop into a spherical vesicle precociously forestalling the further development of the mother-cells of the embryo of which this larval envelope had originally been an organ of protection and of locomotion. Subsidiarily this vesicular shape would contribute towards the retention of the developing egg for a longer time in the maternal genital ducts. And at the same time the possibility would arise of introducing through the wall of this swollen blastocyst not only fluid to increase the swelling, but also nutritive matter to further the growth of the didermic "Anlage" contained in it.

All these circumstances accompanying the transition to an atmospheric environment would at the same time be unquestionable advantages of protection and nutrition to the embryo, such as are already sporadically obtained in certain fishes (*Mustelus*, *Zoarces*, and others). Besides this, however, another advantage might ensue, viz., the possibility of this larval and transitory layer becoming vascularized in aid of a yet more thorough system of nourishment at the expense of the maternal circulatory system.

And it is this what we actually observe in the mammals

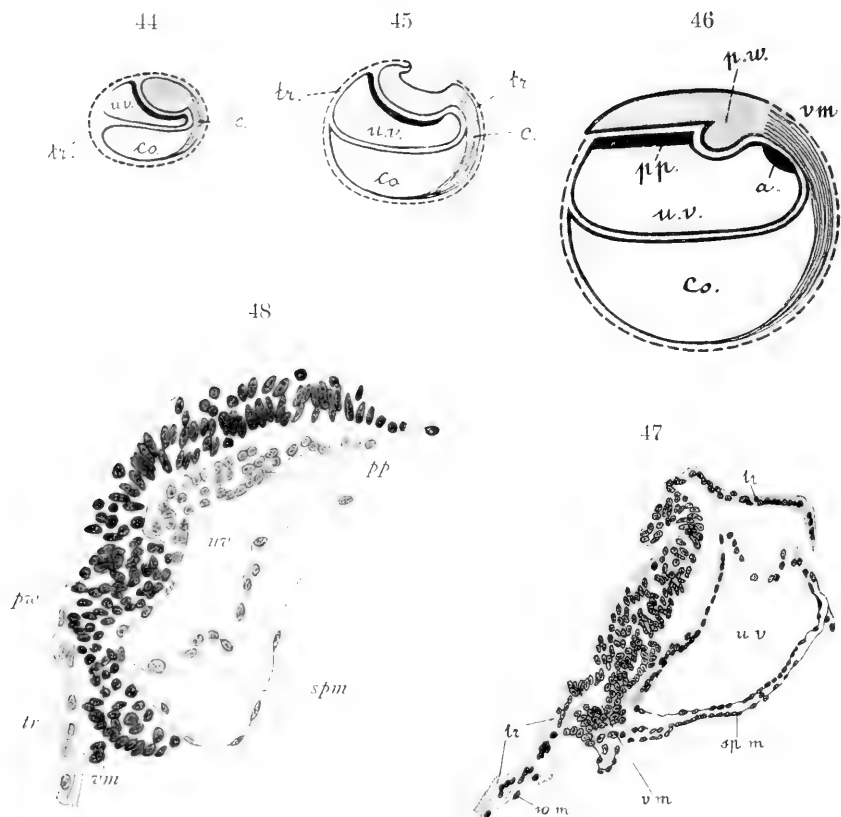
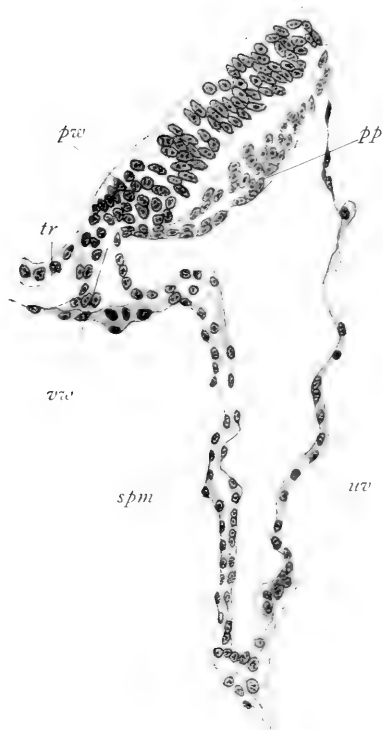
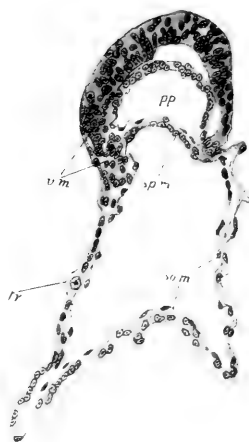


Fig. 44—46. Three diagrams of the aspect of a longitudinal section through a *Tarsius* blastocyst. In Fig. 44 the trophoblast yet covers the embryonic ectoderm. The cavities of the umbilical vesicle (*uv*) and of the extraembryonic coelom *co* in the ventral mesoblast entirely fill up the blastocyst; the connective stalk (*c*) is formed and it is at this spot (cf. Fig. 62) that the attachment of the blastocyst to the maternal tissue comes about. In Fig. 45 the embryonic ectoderm has become exposed to the surface after dehiscence of the trophoblast; the entoderm in the embryonic region has thickened. In Fig. 46 protochordal plate *pp* and protochordal wedge *pv* have become differentiated (cf. Fig. 48); under the stalk of ventral mesoblast the annular region of proliferating entoderm is once more cut longitudinally (*a*); from here the vascularization of the connective stalk proceeds. — Fig. 47. The relative positions of ventral mesoblast (*vm*), trophoblast (*tr*) on its way to leave the embryonic ectoderm uncovered (cf. Fig. 20) and umbilical vesicle in a stage of about the same age as the following figure. — Fig. 48. A somewhat later stage in which a distinct ventral proliferation (*pv*) of the ectoderm fuses with the entodermic proliferation (*pp*) of the entoderm. The protochordal wedge *pv* and the protochordal plate *pp* then become fused (cf. Fig. 52, 98, 99); the ventral mesoblast *vm* springs from the embryonic ectoderm just behind the protochordal wedge. *tr* trophoblast, *spm* splanchnic mesoblast, *uv* umbilical vesicle.

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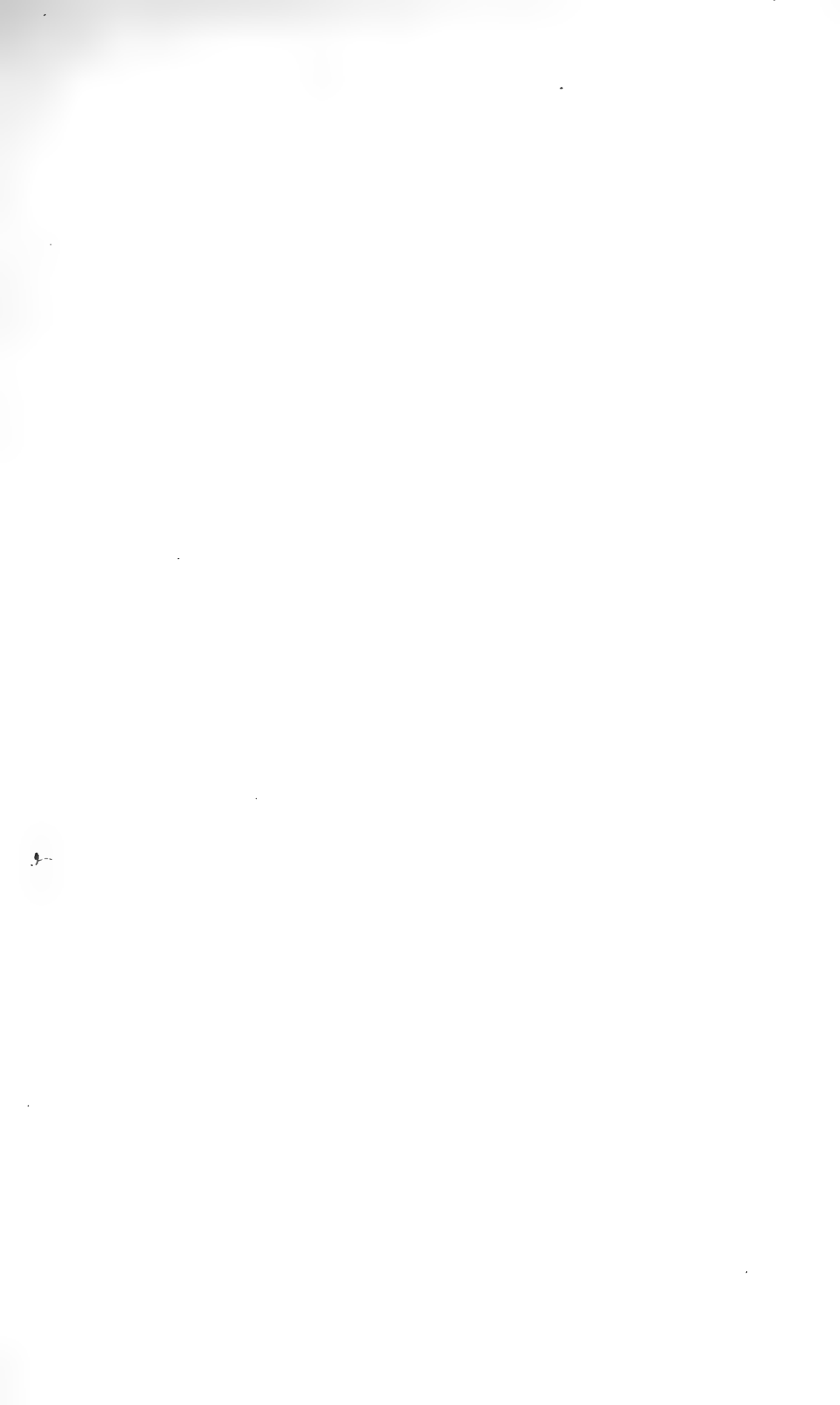
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Fig. 49. Longitudinal section of another *Tarsius* blastocyst in which the protochordal plate *pp* has become fairly established and the protochordal wedge is just in its very earliest phase, more so than in Fig. 48. The ventral mesoblast *vm* arises from the ectoderm, close behind *paw*; the trophoblast *tr*, is independent of both. — Fig. 50. Longitudinal section in about the same stage: the attachment of the blastocyst to the uterine wall commences about at the spot marked *tr*: the corresponding proliferation of trophoblast (*tr*) is not indicated in this figure (cf. Fig. 62); the ventral mesoblast *vm* springing from the ectoderm shows the extraembryonic coelom the wall of which is partly splanchnic (*spm*), partly somatic mesoblast (*som*); *pp* protochordal plate. — Fig. 51. Transverse section of an early blastocyst of about the stage of fig. 46 showing the proliferating protochordal plate.



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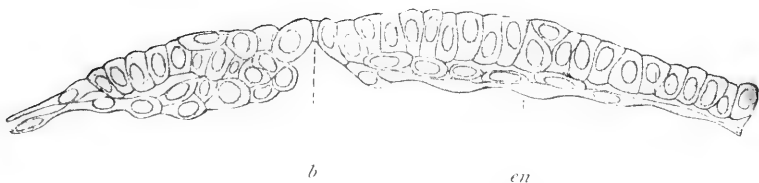


Fig. 52. Longitudinal section of a *Tarsius* embryonic shield in which at the spot where the protochordal wedge *pw* has proliferated downwards a rudimentary attempt at a blastoporic perforation has quite exceptionally arisen. *pp* protochordal plate, *pw* protochordal wedge, *vm* ventral mesoblast, *uv* umbilical vesicle. *ec* embryonic ectoderm: *mcs* mesoblast, springing from protochordal plate. — Fig. 53. The early evanescent blastopore (*b*) of the hedgehog (after Hubrecht, 02). — Fig. 54. The same of the mole (after Heape); *b* blastopore, *en* entoderm.

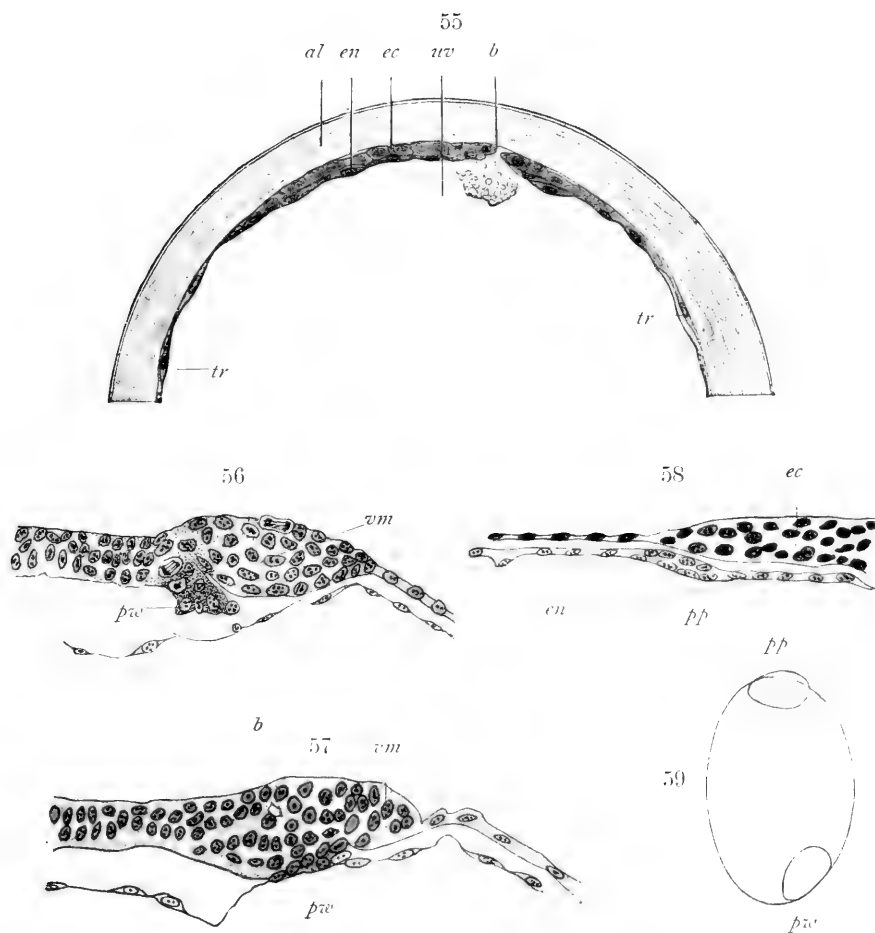


Fig. 55. The same of the opossum (after Selenka); *b* blastopore, *uv* umbilical vesicle, *ec* ectoderm, *en* entoderm, *al* albuminiferous layer, *tr* trophoblast. — Fig. 56, 57 and 58. Three longitudinal sections through an early blastocyst of the shrew (*Sorex*) of which the embryonic shield is traced in the diagram of fig. 59. Fig. 56 and 57 are two succeeding sections through the posterior region where a rudimentary blastopore (*b*) pierces the embryonic shield, separating the proliferating ectodermal region *pw* (protochordal wedge) from the yet further posterior ectodermal region which will give rise to the ventral mesoblast (*vm*), in which the posterior coelom will take its origin in crescent-shape as indicated in diagram 61 and 100. Fig. 58 is the longitudinal section through the entodermal proliferation *pp* of Fig. 59. Fig. 100 gives a longitudinal section through the posterior crescentic coelom of fig. 61. — Fig. 59. Superficial aspect of the early ectodermal shield corresponding to the three preceding figures. The region of the protochordal wedge is indicated by the posterior white, that of the protochordal plate by the anterior shaded space.

from the Didelphia onwards, where either the omphaloidean or the umbilical arteries, or both, serve that purpose.

This then is my interpretation of the phylogenetic phases through which the trophoblast has passed. They cannot be said to be numerous or intricate, nor can the interpretation be looked upon as strained or artificial. The less so, because in all mono- and di-delphic mammals, which have as yet been examined, we do—as was noticed above—encounter a larval envelope—the trophoblast—which surrounds the formative cells of the embryo. Without exception the trophoblast undergoes the series of changes and physiological transformations here sketched, becoming first vesicular, then selective to certain nutritive matter, finally vascularised and locally strongly adhesive to and fused with maternal tissue.

B. ORNITHODELPHIAN MAMMALS AND SAUROPSIDA.

The segmented egg of *Ornithorhynchus* and *Echidna*, the two living representatives of the Ornithodelphia presents itself to us under a totally different aspect, as compared to the other mammals. The ornithodelphian egg does not cleave according to the holoblastic, but to the meroblastic type, and offers numerous points of comparison with that of reptiles and birds. However, our knowledge of it is as yet very scanty and limited to what Caldwell ('87), Semon ('94), and Wilson and Hill ('03, '07) have taught us. The egg is enclosed in a leathery shell. There is no, or hardly any, albumen, and this makes investigation of the earliest stages all the more difficult.

The formative protoplasm, accumulated at the upper pole of the yolk, breaks up into a number of cleavage-cells (Fig. 66) and at a very early stage the outer layer, already distinctly visible as such in the preceding stage (Fig. 67), has spread over the yolk as a membrane of flattened cells with flattened nuclei (Figs. 68 and 69). At the upper pole this layer covers—at the spot where the embryo is going

to be formed—the remains of the cleavage cells not as yet arranged in regular layers. I think we may safely compare this stage in the Ornithodelphian development with that of the higher mammals in which the, as yet undifferentiated embryonic knob is covered by the trophoblast, which has dilated into a vesicle. Although the interpretation here given differs from that of Semon, I feel confident that further and more detailed researches on the development of Monotremes will confirm this hypothesis, as well as the supplementary one which at present is not yet based on observation, viz. that the cells *e.k.* in Figs. 67 and 69, after a time arrange themselves into embryonic ectoderm and entoderm, the latter spreading out radially below the trophoblastic cell-layer, as indicated in Fig. 70. It is particularly to be regretted that the embryonic shield belonging to Semon's Fig. 39 has come to grief, because it would no doubt have settled the point here under discussion.¹

The difference between the Ornithodelphia on one side and between Mono- and Di-delphia on the other would—if the interpretation here given were to be confirmed—consist in the fact that the trophoblast vesicle of the former includes besides an embryonic knob a very considerable amount of food-yolk, the development of which will have gone parallel with the change in the ancestral line from viviparity to oviparity.

Also in the Sauropsida similar phenomena must have occurred of which, however, the traces are yet more difficult to establish than was the case in Ornithodelphia. The trophoblastic vesicle, which is in Ornithodelphia yet comparatively distinct, though as yet imperfectly known, is in many reptiles and birds distinguished with great difficulty from the embryonic shield because the phenomenon of the trophoblastic vesicle opening up at one spot, in order to let

¹ When this paragraph was first written Wilson and Hill's latest extensive researches ('07) had not yet come into my hands. Their figures, here reproduced in the Figs. 68 and 70, seem to fully agree with the hypothetical interpretation here given, before these new facts had come to light.

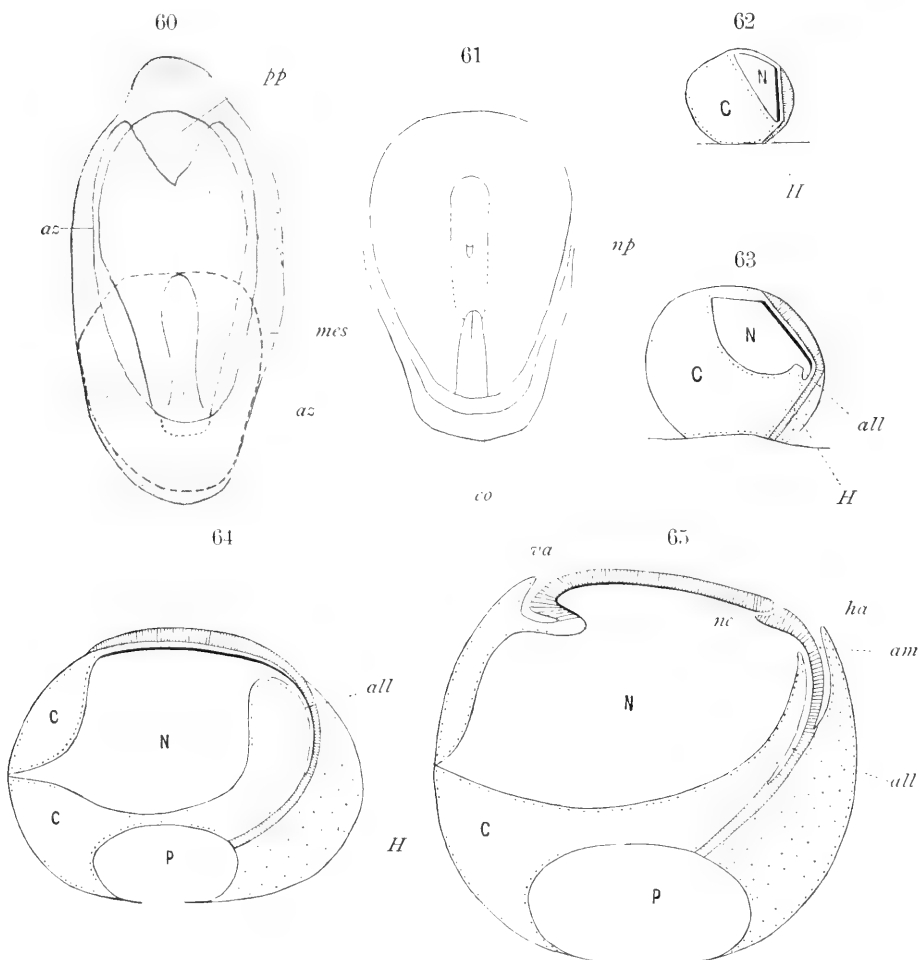


Fig. 60 and 61. Two surface views of a yet later embryonic shield of the shrew. In fig. 60 the annular zone of proliferating entoderm *az* is indicated as well as the primitive streak and the dotted outline *mes* of the mesoblast wings; in fig. 61 the notochord has begun to be formed; a neurenteric pore (*np*) is visible as well as extra embryonic posterior coelom, *co*. (Fig. 56 to 61 after Hubrecht, '90.) — Fig. 62 to 65. Diagrams intended to demonstrate the gradual displacement in Tarsius of the embryonic shield from its original position (62) towards a position diametrically opposite the placenta (65). The zone *a* of fig. 16 is shown in fig. 63 as being at the same time the incipient allantois tube *all*: in fig. 64 and 65 this becomes a posterior, cylindrical continuation of the enteric cavity *N*, lengthening as the embryonic shield travels upwards. In 64 and 65 the placenta *P* has become a considerable trophoblastic proliferation in cushion shape (vide Hubrecht, '99), in Fig. 65 the amnion folds *va*, *ha* and the neurenteric canal *nc* have made their appearance (cf. fig. 99); *c* extraembryonic coelom, *H* connective stalk, *am* amnion (after Hubrecht, '07).

the embryonic ectoderm come to the surface, has become indistinct. It was above shown how perfectly distinct this is in monodelphian and didelphian mammals, and how there can be no doubt of its occurrence in Ornithodelphia (Fig. 70). Still this latter group helps us to explain how it was that it became indistinct and thus unrecognised in Sauropsids.

An outer trophoblastic layer has been described by Mehnert ('94, p. 214), who perfectly recognised its identity with the layer for which in Mammalia I had introduced the name of trophoblast, but who has created confusion by nevertheless proposing the new name of teloderm¹ (Grenzblatt), and greater confusion yet by comparing heterogenous cell-layers as I will yet further indicate. Mehnert describes in detail how in the embryo of *Emys lutaria* the outer germ layer becomes didermic and produces two layers that are totally different from each other, of which the deeper layer furnishes the material for the definite epithelium of the tortoise and represents the primitive epidermis, whereas the outer layer of flattened cells, the trophoblast (Mehnert's teloderm), should be looked upon as a supra-epithelial layer. According to Mehnert the trophoblast can be quite easily separated from the epiderm (l. c., p. 213, Pl. IX, Fig. 8).

The growth of the trophoblast is said to be dissociated from that of the deeper epithelial layer. Mehnert claims to have established (on the authority of Mitsukuri's ['93] figures) the presence of a trophoblast in *Clemmys japonica* and in *Trionyx japonica*, in *Lacerta muralis*, *Tropidonotus*, and for birds in the duck, the chick, *Larus*, *Sterna*, *Podiceps*, *Buteo*, *Aegialitis*, *Hirundo*, *Luscinia*, and others. Now I must begin

¹ The reason he gives for substituting a new name and not applying the name of trophoblast is, "that it has not been proved that those cells participate in the first place towards the nutritive processes of the embryo." In this he is in full contradiction to Schauinsland ('03, p. 33) who holds it to be "sehr wahrscheinlich" that these very cells have a nutritive significance in reptiles. In the Mammalia, where the layer is ever so much more conspicuous, its phagocytic significance has been proved; but even if it had not, this seems hardly to justify Mehnert for over-burdening scientific nomenclature by the creation of a superfluous synonym.

by disclaiming the greater number of these cases. I feel convinced that in certain of the cases observed Mehnert and Mitsukuri have seen what is really the rudimentary plasmodiotrophoblast of reptiles, but that in others the first-named author has been misled and has confused what is really a superficial layer (distantly comparable to a mammalian epitrichial layer) of later embryonic phases with trophoblastic elements that can only be noticed in certain early phases. I have published this disclaimer more than ten years ago ('95, p. 27, Anmerkung); I can here only repeat it. A real Reptilian trophoblast can, I think, be clearly detected in Mitsukuri's ('90) Fig. 59 of *Clemmys*, where we find a separate cell-layer of flattened elements accompanying the amnionfolds on their outer surface. This layer is not continued on the inner surface of the amnionfolds as Mehnert will have it in his case of *Emys lutaria*. Also in his coloured figures (l. c., 30a—37a) Mitsukuri seems to indicate, by a different tint of red, that he did not (as does Mehnert) see any continuity between this outer trophoblastic layer and the inner lining of the amnion.

If we were to adopt Mehnert's view—as I have perhaps been inclined to do more than I was justified to in 1895—then we would have to look not only upon the inner layer of the amnion as trophoblastic, but also upon the covering layer he describes in the duck, which forms a continuous suprapithelial stratum both on the back and on the ventral surface of the embryo; and a comparison with what we have above described for the mammals ought to make us diffident in accepting this view as the real interpretation.¹

¹ It must be borne in mind that the phenomena here discussed are as yet only very partially known. And if we consider the very various methods which we have discussed above (p. 11), according to which the mammalian trophoblast disappears above the embryonic shield, we may also expect variations in the Sauropsida. If we suppose that an arrangement like that in the rabbit and other rodents (Figs. 15 and 2B) where the Rauber Deckschicht remains distinct for yet a longish time, were yet further protracted, we might obtain a state of things as that which is described by Mehnert for *Emys* and certain other forms. I should not mention this if it were not

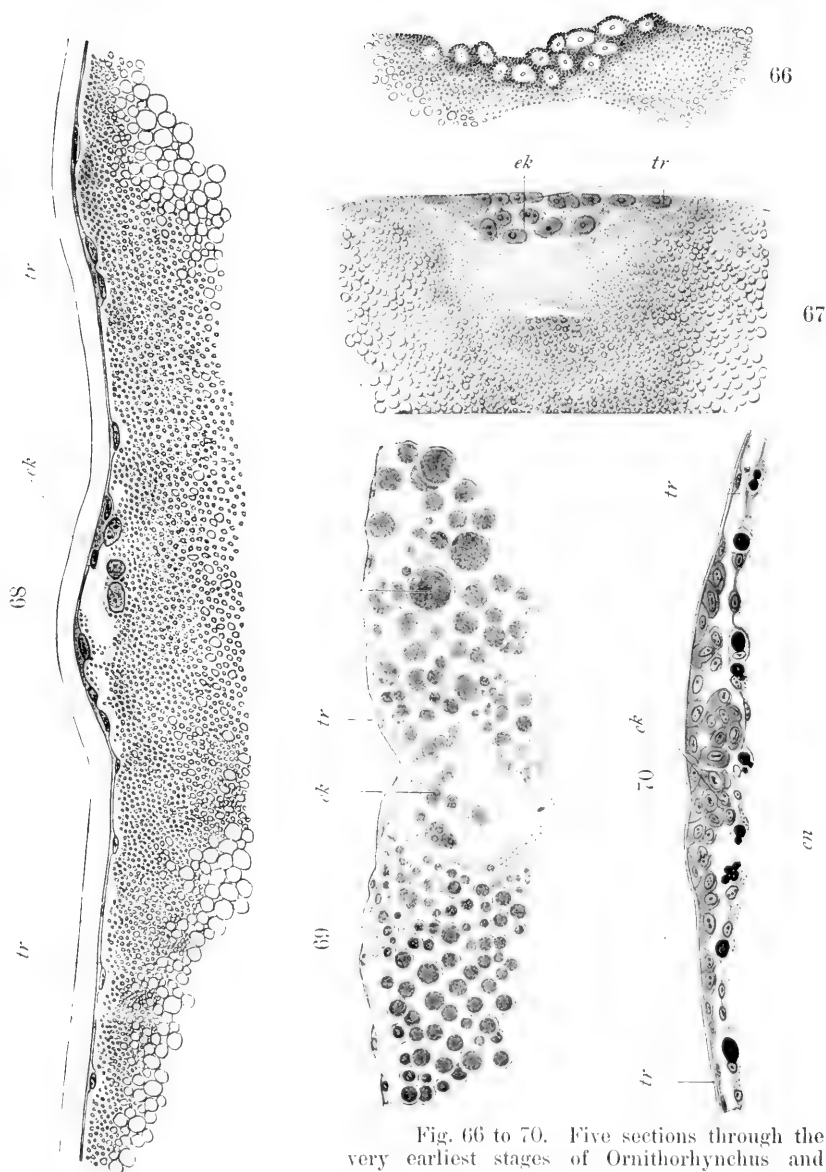


Fig. 66 to 70. Five sections through the very earliest stages of *Ornithorhynchus* and *Echidna*. Fig. 66 earliest cleavage stage. Fig. 67 visible separation of trophoblast cells *tr* and of mothercells of the embryonic knob, *ek*. Fig. 68 and 69 this separation is yet far more clearly established: the trophoblast *tr* having travelled much further over the yolk surface, the embryonic knob (*ek*) being partly imbedded in the yolk; Fig. 70 a yet further stage in which ecto- and entoderm (*ec* and *en*) have become differentiated by delamination and in which the ectoderm has come to the surface; *tr* trophoblast. (Fig. 66, 68, 70 after Wilson and Hill, '07; Fig. 67, 69 after Semon, '94.)

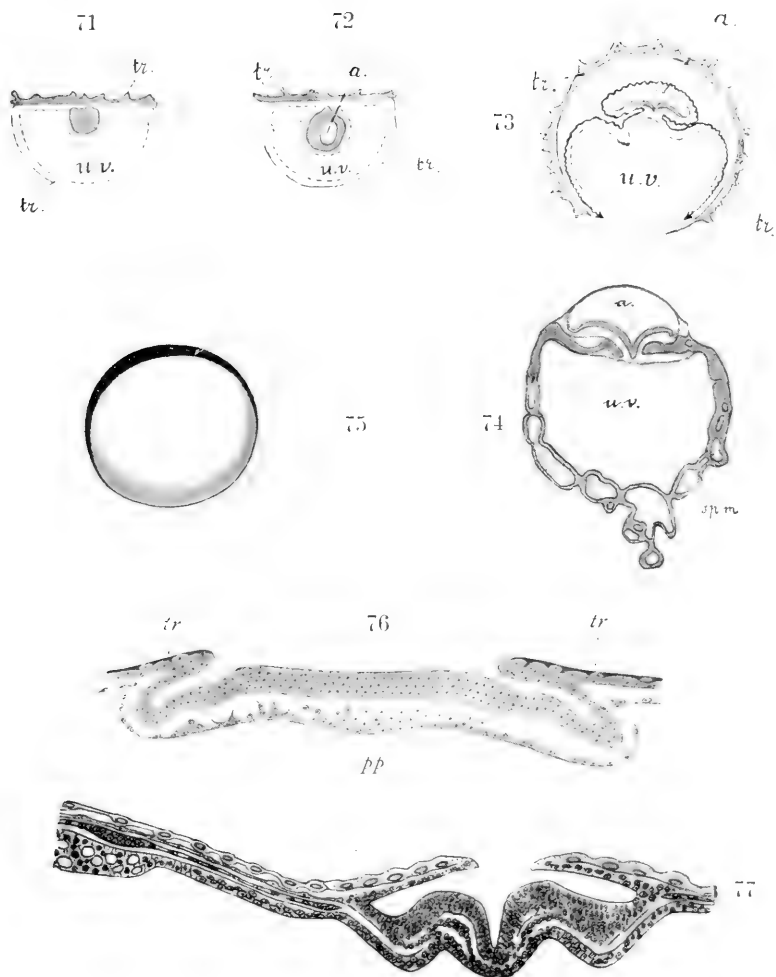
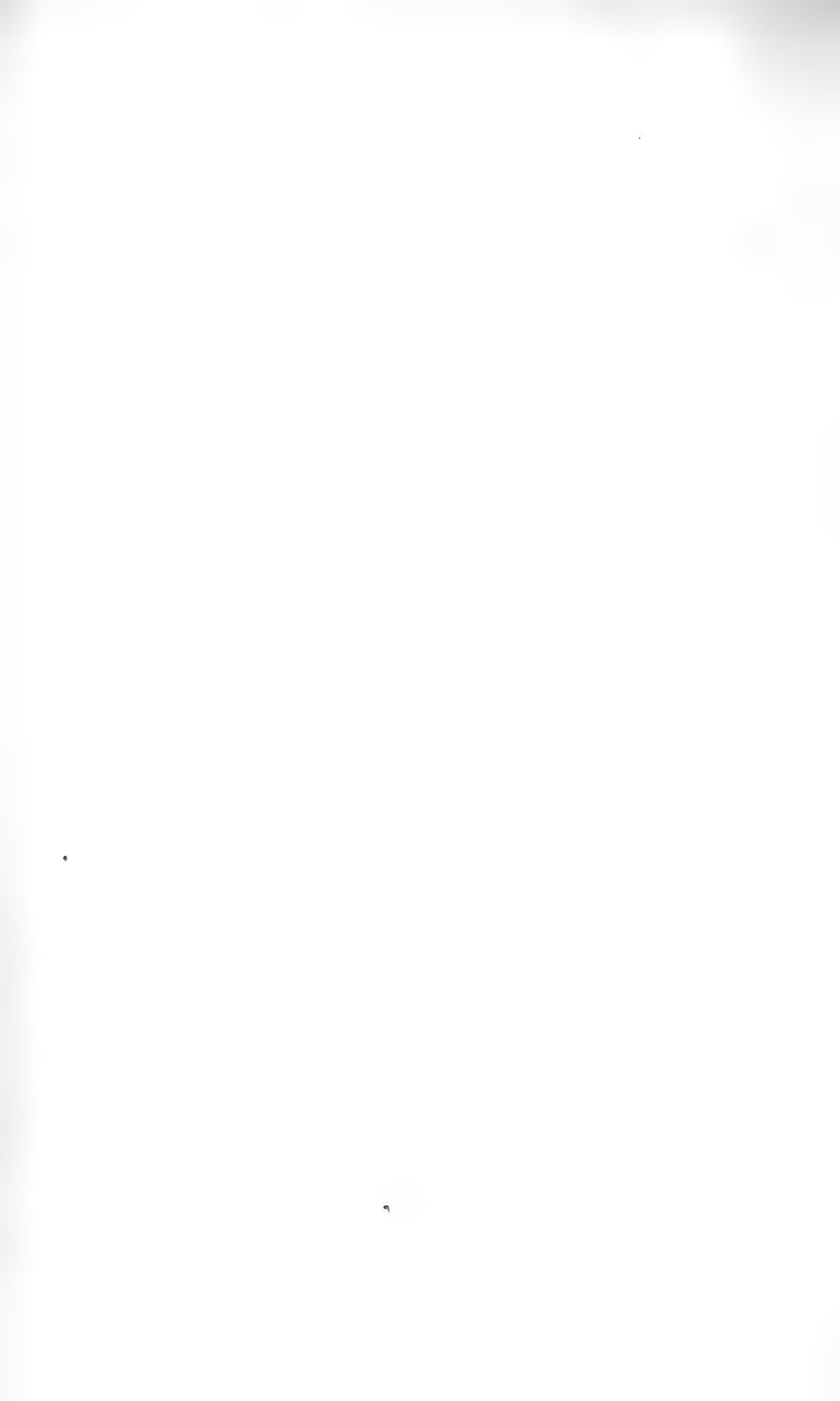


Fig. 71 to 73. Three figures of sections through the blastocyst of the frugiverous bat *Pteropus* (after Selenka, Göhre, '92). In fig. 71 the embryonic ectoderm is yet a solid cellmass, in fig. 72 an amnion cavity (*a*) has appeared within it, in fig. 73 the final relations between trophoblast (*tr*), amnion *a*, embryo and umbilical vesicle (*u.v*) are established. — Fig. 74. Transverse section through amnion *a*, embryo and umbilical vesicle *u.v* of *Hyllobates* (after Selenka, '00). *spm* the splanchnic mesoblast on the umbilical vesicle (*u.v*) which carries a very dense net of thickened venae in which haematopoietic processes occur. — Fig. 75. Surface view of the amnion-fold of *Chamaeleo*. — Fig. 76. The same in transverse section, with proliferation of the anterior entoderm (*pp*). *tr* trophoblast separated into two layers. — Fig. 77. Transverse section of an embryo of *Sphenodon* with amnion nearly closed. The trophoblast is double layered. Fig. 75 to 77 after Schauinsland, '03.



A second author in whose investigations a reptilian plasmoditrophoblast has come to light is Schauinsland ('03). In his figures of the young *Chamaeleo* (Fig. 76) and *Sphenodon*-embryo (Fig. 77), we notice that the rising folds of ectoderm, which are the first indications of the separate existence of amnion and serosa, are covered, externally, by a layer of varying thickness. The presence of this layer seems to me indicative of a similar process in reptiles as was noticed in mammals, viz. a differentiation of the region outside of the ectodermal shield (as such we encounter the trophoblast after the embryonic ectoderm has been interpolated in it) into a superficial and a deeper layer (plasmodi- and cytotrophoblast of v. Beneden and Hubrecht). And this differentiation arouses suspicion, further confirmed by the sharp distinction at the free border of the amnion fold between outer and inner layer (Figs. 76 and 77, that in reptiles the case may stand as in bats (Fig. 8a), and in the hedgehog (Fig. 38) where the outer surface of the amnion-fold is trophoblastic, whereas the inner is an upgrowth of the ectodermal shield (see also p. 77) and Duval ('99, figs. 96, 102 and 117). The trophoblast of *Sphenodon* and *Chamaeleo* would thus be more than one cell thick even before the somatic mesoderm has made a diplotrophoblast of it. This trophoblast does not contribute to line the inner surface of the amnion cavity. Here only the embryonic ectoderm (see pp. 76—78) comes into play. In this important respect Schauinsland thus sides, although not himself discussing the merits of the problem (which was not before his mind), with Mitsukuri and not with Mehnert. In *Chamaeleon* of which Schauinsland gives good illustrations ('03, Pl. 26, figs. 184—186), which are very indifferently reproduced in Hertwig I, 2, p. 194, the same phenomenon is observed with quite as much distinctness (Fig. 76). After the amnion has closed in the very primitive fashion characteristic for *Chamaeleon* (Fig. 75) the "membrana serosa" consists of a double layer of trophoblast (Fig. 76).

desirable, from the very first, to keep an open eye for all the different possibilities that may help to elucidate these difficult points.

The facts above cited force us to the conclusion that, before the formation of the amnion in *Sphenodon* and in *Chamaeleo* begins, there must exist on the surface of the blastocyst a circular delimitation of a central region—what would be the actual embryonic shield of mammals—from a peripheral trophoblastic region. This delimitation is clearly indicated in another of Schauinsland's figures (Pl. 46, fig. 117) for *Sphenodon* not reproduced in Hertwig, but here reproduced in Fig. 78. In Schauinsland's text ('03, p. 142) this is noticed in the following words:—"As it was repeatedly noticed (the trophoblast-cells) do not spread over the embryo proper, and thus the extra-embryonic and the embryonic portion of the ectodermal blastoderm can be sharply distinguished from each other."

If we now restrict ourselves to the three cases here cited, a tortoise (*Mitsukuri*), *Sphenodon*, and the *chamaeleon* (*Schauinsland*), and purposely leave out of consideration all Mehnert's cases, then we have three Sauropsida in which clear indications are noticeable that the mammalian trophoblast is after all also present in the Sauropsida.

Besides these indications there is, however, a strong *à priori* probability that views which are applicable to the embryonic membranes of mammals ought also to fit in with Sauropsids that have—because of these membranes—always stood in closer connection with the mammalia than with the lower vertebrates.

And we should not lose view of the fact that the comparison of Elasmobranch with Sauropsid ontogeny has always shown this incisive difference that there was never a *membrana serosa* nor an amnion in the former, so that a direct comparison in these two types of the process of the gradual inclosure of the yolk by radial expansion from the ectodermic shield was tainted by suspicion from the beginning: the whole of the serous membrane and the amnion being shed at birth in birds, reptiles and mammals; these being, in fact, larval layers.

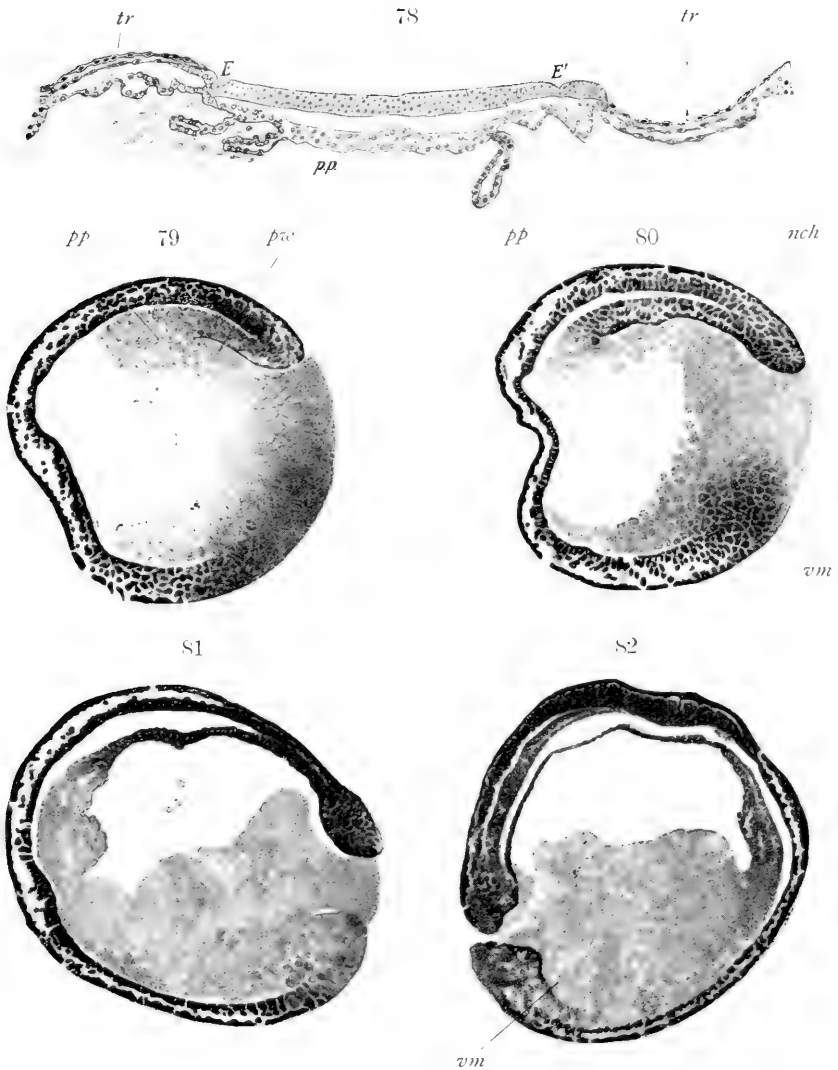


Fig. 78. Another transverse section of *Sphenodon* (after Schauinsland, '03) to show the differentiation of the twolayered trophoblast *tr* as against the ectodermal shield *EE'*; *pp* protochordal plate. — Fig. 79 to 82. Four longitudinal sections of frog-embryos (after Brachet, '02). In Fig. 79 protochordal plate *pp* and protochordal wedge *pw* have become differentiated; in Fig. 80 the notochord (*nch*) is further developed and the ventral mesoblast *vm* makes its appearance; in Fig. 81 the segmentation cavity has coalesced with the enteric cavity that has become visible during notogenesis; in Fig. 82 notochord, somites and gut are formed, headfold has become visible, ventral mesoblast *vm* develops below and behind the entoderm cells

And now that the interpretation of the facts in mammals has become comparatively easy (see also Chap. III) we should not shrink from resolutely interpreting the Sauropsidan development along the same lines.

A comparison of my own figures for early *Erinaceus* ('89) and of van Beneden's ('99) for early *Vespertilio* blastocysts with the figures above referred to of Schauinsland and Mitsukuri convinces us of the possibility of looking upon the double layer outside the formative ectoderm—say of *Sphenodon*—as a duplication of the trophoblast. The two mammalian genera above mentioned, as also *Sorex* and others, show a duplication and even a more considerable thickening yet of the trophoblast immediately outside the embryonic ectoderm. And so it would not be a very strained assumption to say that in reptiles and birds—in which as we have seen Schauinsland admits of a sharp line of demarcation between the trophoblast and the embryonic shield on the surface (l. c., p. 142)—both layers that are outside of this line of demarcation are trophoblast-cells separated in an outer flattened and a deeper columnar layer. Even of this differentiation in shape the mammals offer the counterpart, as is seen, to the left side in Figs. 8 and 8a of van Beneden's ('99) early bats and Figs. 35—37 of the hedgehog here given. We will, moreover, see in Chap. V that the trophoblast often differentiates into two layers that are known as the cytotrophoblast and the plasmoditrophoblast. And so the assumption here advocated would oblige us to conclude that, in birds and reptiles, a circular patch of embryonic cells was separated—not visibly but potentially—from a peripheral region of trophoblast cells just as we have established this for *Tupaja*, *Tarsius*, and others, in which—after the embryonic shield has opened out—it is no longer possible to distinguish the line of demarcation between trophoblast cells and embryonic ectoderm cells, although we have noticed its actual existence in the successive ontogenetic stages. In most Sauropsida ontogeny would no longer clearly reveal this difference, but still the mutual relations would be the same, and exceptionally favourable cases as here described and figured (*Clemmys*, *Sphenodon*, *Chamæleo*) would be all the more welcome confirmations.

Physiologically the outer layer of the serosa of Sauropsids is recognised to have undoubtedly (see p. 21, footnote) certain properties which we also encounter in the proliferating trophoblast of mammals. There is, for example, a very marked proliferation in the outer layer of *Seps*, a viviparous lizard in which Studiati, Giacomini ('91), and others have described both an allantoidian and an omphaloidean contact (placentation) between the serosa and the maternal tissues.

Similarly the action of the serosa of the chick in the region where Duval has described the "organe placentaire" gives rise to the same considerations.

But more extensive investigations *ad hoc* will have to be undertaken before the isolated cases of the Reptilia above noticed will have obtained sufficient lateral support to serve as a starting-point on which a theory on the modification of the trophoblast in the Sauropsida—simultaneously with the formation of an eggshell, etc.—may be based.¹

Of the part played by the Sauropsidan trophoblast in the formation of the amnion we will have to speak in another chapter. Suffice it to add that no data are as yet available to determine the exact moment at which the plasmodi-trophoblast becomes distinguishable in the above-mentioned genera. Neither Mitsukuri nor Schauinsland give any indications. Furthermore, it would be important to know whether ontogeny gives any clue which would permit a guess as to the question whether the trophoblast has, in the viviparous ancestors of the Sauropsida, been as early differentiated from the remaining cleavage cells as is the case in mammals,² or whether the differentiation has only set in later as we find in the case of those Amphibia and fishes in which traces of an outer larval layer are also present, and which we will more fully discuss in the last paragraph of the next chapter.

C. ICHTHYOPSIDS.

In the paragraphs A and B of this chapter we have attempted to show that beside the ectoderm and entoderm, which by delamination establish the gastrula stage of mammals and Sauropsids, there exists yet another very early cell-layer

¹ Recently Eternod has published an article, "La Gastrule dans la série animale," in the 'Bull. Soc. Vaud. Sc. Nat.,' 1906. 5e sér., vol. 42, in which, in text-fig. 16 and in fig. 26 on pl. 13, he attempts to homologise parts that are in no way homologous, if we look upon the early developmental processes of Mammals and Sauropsids in the light above advocated. Eternod's views have already been successfully protested against by Schlater ('Anat. Anz.,' Bd. 31, p. 31). The latter author himself misses the mark, however, when he says that "die epiblastische Schicht der Sauropsiden-keimblase der über die Grenzen der Keimscheibe hinausgewachsene embryonale Epiblast ist." The secondary degenerative stages of the trophoblast are here wholly misunderstood.

² The researches, above alluded to (pp. 12 and 20), of Wilson and Hill seem to imply that in *Ornithodelphia* we have yet an important intermediate stage, in which it is indeed possible, notwithstanding the yolk accumulation, to distinguish the trophoblast from the mother-cells of the embryonic knob. Semon's ('94) figs. 33 and 34 allow of a similar interpretation.

to which the name of trophoblast has been given. This layer, phylogenetically subordinated to the ectoderm, was looked upon as a differentiation of the same order as the outer larval layer which in certain Nemertines, Gephyreans, and other worms often serves as a temporary envelope that is stripped off when the animal attains to a certain stage of development. In a later chapter it will be discussed whether the different foetal envelopes of the Amniota allantoides may not be brought into genetic relation with this layer, and whether we might be justified in thus tracing the foetal envelopes of the higher vertebrates as far back as the invertebrate ancestors provided with an ectodermal larval investment (Larvenhülle).

It would appear at first sight probable that in the Anamnia, Anallantoidea (i. e. in the Ichthyopsids) traces of this larval cell-layer should not be met with, and that this very absence would help to explain the fact that here no amnion develops. However, the chance that the intrinsic differences between say Amphibia and Reptiles are not so incisive as this separation of the vertebrates in Amniota and Anamnia would make us believe, should also yet receive our consideration. And it is in this light that I intend to look upon the fact that in many amphibia certain ontogenetic stages reveal the presence of what has been called the "Deckschicht" of the larva. Numerous figures successively published by different authors show the extent to which such a layer has been actually observed. It should at the same time be noticed that in several other genera no trace of it has been found.

The more remarkable circumstance is, however, this—that not only in Amphibia such a "Deckschicht" makes its occasional appearance, but that similarly it is noticed during the development of certain Dipnoi and Ganoids (Fig. 87), and both more constantly and more unquestionably during that of all the Teleosts (Fig. 89) of which up to now the early development has been traced. Of these different groups the "Deckschicht" is here figured after the publications of different authors on the subject, and I will not here enter into further details, contenting myself with having shown that it is a

general feature in the development of Teleostomes, Dipnoi, and Amphibia.

Suppose for a moment that we are justified in looking upon the Deckschicht of Amphibia and Teleostomes as being in reality homologous to the trophoblast of Mammalia and Sauropsids—homologous at least in that sense that what is a very active and most important layer during the development of the viviparous mammals is only a temporary, evanescent arrangement in the Ichthyopsids—then we must at the same time ask ourselves: is this homology, perhaps, indicative of an error into which we may have fallen when adopting Milne Edwards' distinction of the vertebrates in Anamnia and Amniota? And should we not reconsider whether and how this error can be readjusted?

At all events the Elasmobranchs, the Cyclostomes and Amphioxus show in their early development no traces of a Deckschicht and—as we shall see in a later chapter—no traces of other organs which are characteristic for the other vertebrates.

In this chapter I had to point to these facts; in Chapter III, p. 81, they will be more fully discussed, as also in Chapter VI, p. 150.

CHAPTER II. FURTHER DEVELOPMENT OF THE TWO GERM-LAYERS OF THE VERTEBRATES UP TO THE APPEARANCE OF THE SOMITES.

I. MAMMALIA (MONO- AND DI-DELPHIA).

1. Developmental Processes in the Entoderm.

The participation of the entoderm towards the formation of tissue between the two primary layers in Mammals is denied by very high authorities as Kölliker, Selenka, Ziegler, Keibel, and others, who hold that material for mesoblastic

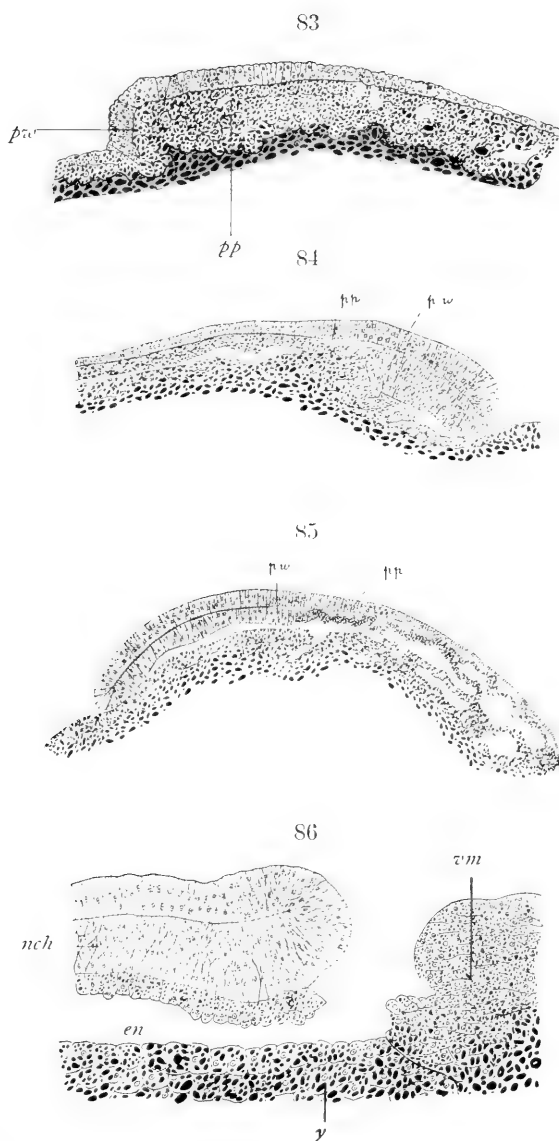
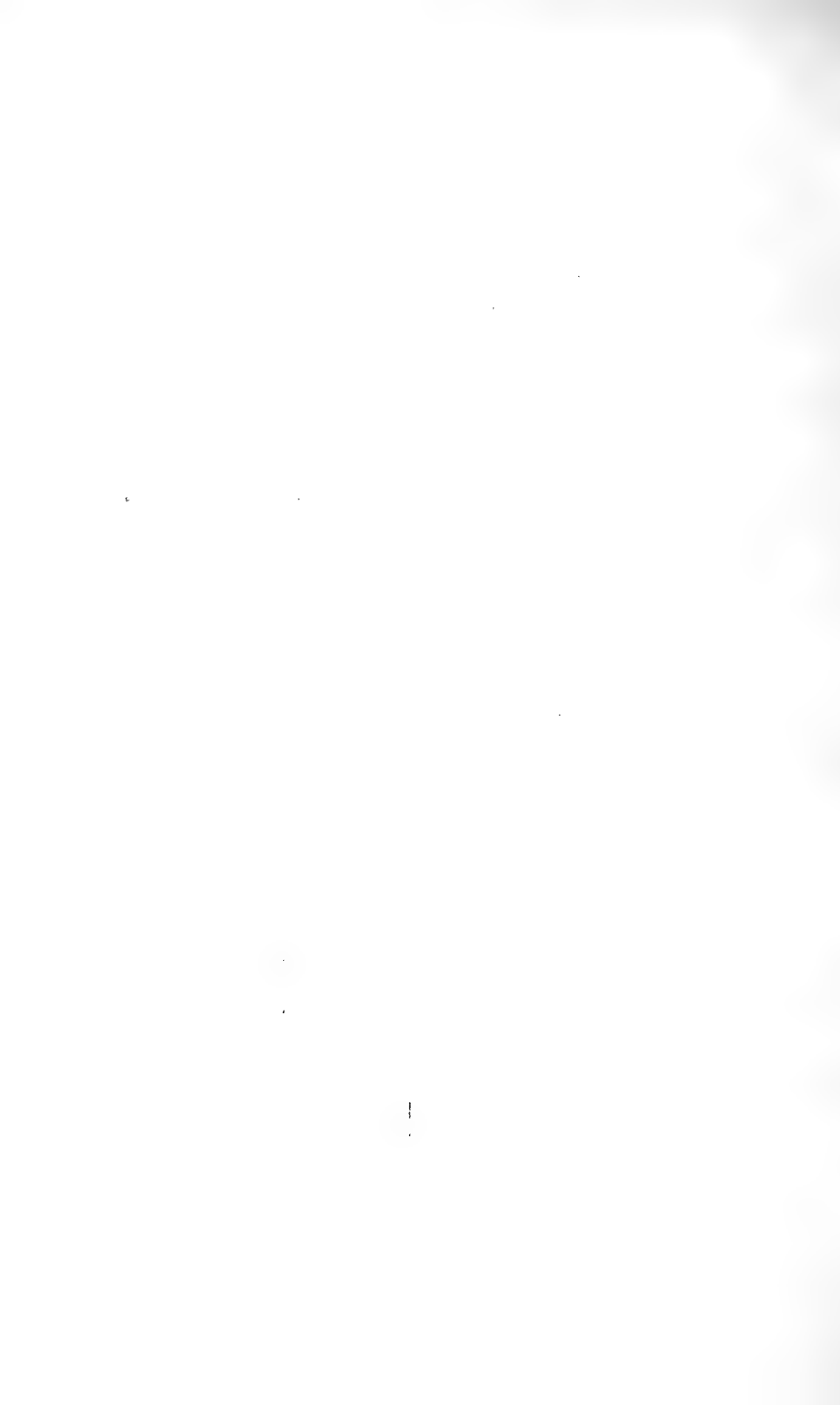


Fig. 83 to 86. Four longitudinal sections of *Hypogeophis* (after Brauer, '97). In Fig. 83 the downward proliferation of the ectoderm (*pw* protochordal wedge) commences to fuse with the entodermic protochordal plate *pp*. In Fig. 84 the notogenesis has proceeded further; in Fig. 85 the segmentation cavity has coalesced with the enteric cavity; in Fig. 86 the ventral mesoblast *vm* has also made its appearance and the entoderm *en* has spread below the notochord *nch*, *y* yolk.



structures is budded off only from the primitive streak, and who—some of them at least—even wish to derive the vascular system and the blood from the same source. Mesenchyme formation, so sharply distinguished by O. Hertwig from mesoblast formation (see his 'Lehrbuch,' ed. 1906, p. 218) is by many authors held to be of no significance whatever in mammals, although Bonnet, in his investigations on the sheep's development ('82, '89), has attempted to stem that current of thought in demonstrating for the sheep that the vascular region on the yolk-sac is a direct derivate of local proliferation of the entoderm. In his later publications on the dog, however, Bonnet has for that mammal denied the presence of a similar process, although from his plates ('01, Pls. XVIII, XIX; fig. 6, and many others) another conclusion might certainly be drawn (Figs. 91 and 92). On the contrary for *Sorex* and *Tupaja* (as yet unpublished) the genesis of mesenchyme out of entoderm has been fully confirmed by myself, and the region in which the participation of the entoderm towards the formation of blood-vessels and blood occurs, has been figured in detail by me ('90, Figs. 58, 61). When seen from above the aspect is such as to warrant the designation of this region by the name of the annular zone of mesoblast-producing entoderm of the shrew and of *Tupaja*.

Since then the battle has been raging concerning this very difficult and yet very important question of comparative embryology round which many problems, connected with the interpretation of the germinal layers and the significance of mesoblast, centre.

Only very lately Rückert has given a remarkable digest—in co-operation with Mollier—in Hertwig's 'Handbuch,' Vol. I, p. 1244—1260, in which—starting from careful investigations—he draws important conclusions concerning blood-formation in all the Vertebrates, that go far to demolish part of the theoretical views held by Rabl on mesoblast formation, which latter have been largely accepted by the great majority of embryologists.

I need not here enter into a detailed exposition of this controversy, now that it has been so carefully done in the chapter just mentioned on "die erste Entstehung der Gefäße und des Blutes bei Wirbeltieren," in Hertwig's 'Manual.'

But I will pass on to a full description of what has already been observed and described in different mammals commencing with what, in 1890, I have called—

a. The Protochordal Plate.—This structure has at first been more or less ignored by many embryologists, later its significance has been recognised, but it has then been designated by a different name (Bonnet, '01, E.P.); this time I hope to establish definitely that I was not only justified to distinguish this protochordal plate as an independent anterior source of mesoblast in mammals, but that we ought henceforth to admit its presence under varied aspects also in Sauropsids and Ichthyopsids, as I will point out hereafter.

For mammals we have in the preceding paragraph described how in the didermic stage the entoderm cells under the ectodermal shield are considerably more massive than those that clothe the inner surface of the trophoblast, the latter being flattened and further apart. Figs. 8a, 14, 18, 30, 31 and 36 testify to this. As the didermic blastocyst increases in size there is a very marked phenomenon of further increase coupled with proliferating growth in that portion of the entoderm that lies under what will later be the anterior portion of the embryonic shield. I here re-figure this for *Sorex* after my own (Figs. 58, 59) for the sheep and dog, after Bonnet's (Figs. 91, 92) publications, and I add new figures indicating the same phenomenon in *Tarsius* (Figs. 48, 49, 50 and 51) *Galeopithecus* (Figs. 18, 42). For the pig it has been figured, although not viewed in this light, by Keibel ('93, figs. 21—23).¹

¹ Keibel interprets his figures differently, and did not, in the paper above referred to, recognise the protochordal plate as a source of mesoblast, such as I had defined it three years before. Still the figures here cited leave no doubt of its existence in the pig.

In *Sorex* it was particularly interesting to be able to establish the independence of this early proliferation from any further source of mesoblast, although very soon after, the annular zone of mesoblast-producing entoderm connects this early protochordal plate with the mesoblast-producing regions at the hinder end of the embryonic shield. Seen from above this early phase is pictured in Figs. 59 and 60.

The entodermal proliferation here described has, in its earliest phases, the aspect of a mere thickening of the lower germ-layer, but very soon that aspect changes, and we notice that certain of the proliferated cells break away from their place of origin, and take up a situation between the two germ-layers. The extent to which the invasion by these mesenchyme cells of the space between ectoderm and entoderm spreads cannot always be very strictly determined for two reasons. Firstly, because the annular zone of mesoblast-producing entoderm, which becomes confluent with the protochordal plate (Fig. 60), starts its activity almost simultaneously, though, as Fig. 59 shows, just a bit later; secondly, because another invasion of this space—starting from the ectoderm—also begins to form about this time, and will be described below.

At a very early moment the cells derived from these three different sources intermingle, and it will prove a most intricate problem, which up to now has not yet been fully nor satisfactorily solved, nay, not even approached, to make out from which of the three starting points the various organic tissues have ultimately been derived.

In *Sorex* this was possible to some extent at a very early stage, because the anterior entodermal proliferation producing mesenchyme cells is inaugurated somewhat earlier than the process which starts from the posterior half of the ectodermal shield. In my paper on *Sorex* ('90) I have been able to sufficiently distinguish these early phenomena, although fully recognising that after a time further discrimination becomes impossible. This latter fact may have contributed to bring so many of the best modern embryolo-

gists to follow Kölliker in his negation of the participation of the entoderm towards the production of mesoblast.

In *Tarsius* the distinction of the mesenchyme (derived from the entoderm) from other mesoblast-cells between the two germ layers is hardly feasible even in the earliest stages, because here the source of early ectodermic mesoblast at the hinder end of the ectodermic shield is in full flow at a very early period in consequence of the presence from the very outset of mesoblastic tissue, which I have called the ventral mesoblast. It forms a sac, partly applied against the umbilical vesicle from the very first, and encloses an extra embryonic coelomic space, which is thus present at a very much earlier moment than in other mammals with the exception of man and monkeys. Part of this ventral mesoblast will gradually become the connective stalk (*Haftstiel*, *Bauchstiel*) by which the embryo will be in vascular connection with the placenta, and which will be fully discussed in a later chapter. But in this same *Tarsius* the entodermal proliferation above described for *Sorex*, and which I will continue to designate as the protochordal plate is all the more evident. It is figured in diagrams 48, 49, 50, and 51. The entoderm has here become two or three cell-layers thick. This region corresponds to what will later be the very front part of the head of the embryo, before the primordium of the heart has yet been folded in under that of the brain.

As to other mammals I do not dispose of quite as extensive data as for *Sorex* and *Tarsius*, but there is no doubt if we also consult the results of other investigators—even of those who deny the participation of the entoderm towards the formation of mesoblast—that this thickening of the entoderm occurs in all mammals. For *Erinaceus*, *Gymnura*, *Talpa*, and *Tupaja* I possess numerous convincing preparations already mentioned above. Also for *Manis*, *Galeopithecus*, *Sciurus*, *Mus*, *Lepus*. Several of these are here figured (Figs. 18, 37, 42). For the dog Bonnet gives very unmistakable illustrations ('01, Figs. 11—13, 31, 32), although he substitutes the name "*Ergänzungsplatte*" for the older

one of "protochordal plate." Also in one of Assheton's papers ('96, Pl. 20, figs. 17 and 18) the author clearly figures the proliferating region in the entoderm here referred to.

b. The Annular Zone of Proliferation.—How the hind end of this entodermal protochordal plate comes to fuse with the front portion of a median ectodermal down-growth of the ectodermal shield I have described for *Tarsius* in a former paper (1902). It will again be discussed further down. It is, however, necessary first to establish a fact already formerly insisted upon both by Bonnet ('84) and myself ('90), viz., that when once the protochordal plate has made its appearance as a median, mesenchyme producing spot in the entoderm, the same mesenchyme producing properties become evident in peripheral regions of the entoderm. These regions have been named by Bonnet for the sheep the "Mesoblasthof"; shortly afterwards I have described them ('90) for the shrew as an elongated ringshaped zone of entoderm which is situated under and somewhat outside the border of the ectodermal shield (Fig. 60), and which, slanting backwards from the protochordal plate both right and left, meets under the hinder part of the shield in the region where the mesoblast has acquired that median thickening which is known as the primitive streak, continued in the Primates into the connective stalk (Haftstiel).

The presence of such an annular zone of mesenchyme producing entoderm has been very emphatically denied by such embryologists as Rabl, Keibel, and others, and in O. Hertwig's latest manual he makes no mention whatever of it in the chapter on the "Lehre von den Keimblättern." This is all the more to be wondered at, because we shall see that also in lower vertebrates a similar participation of the entoderm towards mesenchyme formation can as little be denied. It seems to me that the energy with which these facts are ignored must have its origin in the strength of certain theoretical considerations with which a multiple origin of mesoderm¹ would clash.

¹ For myself, I have on another occasion ('02, p. 84) expressed my symbol.

There is no doubt that to a great extent the mesenchyme here described contributes towards the formation of blood-vessels and blood. The protochordal plate furnishes the endothelium of the heart, as I have elsewhere demonstrated for *Tarsius* ('02, Pl. IX, fig. 73, *a* and *b*), the annular zone produces the material for the area vasculosa on the umbilical vesicle. To that effect mesenchyme cells, which originated at an early stage in the annular region here alluded to, migrate over the surface of the umbilical vesicle and come to be situated between the layer of entoderm which forms its inner, and of splanchnic mesoderm which sooner (*Primates*) or later (other mammals) forms the outer wall of this vesicle. Besides these lateral portions of the annular zone the hinder portion of it, situated diametrically opposite to the protochordal plate, has yet an important part to play in the formation of blood-vessels and blood. From it the vascularisation of the *Haftstiel* of the *Primates* is derived. From the distal end of this connective stalk vessels irradiate towards the whole inner surface of the diplotrophoblast (man and anthropomorphæ) or only towards a restricted circular part of it (*Tarsius*). This vascularisation must phylogenetically have preceded (as we will discuss later on) that which comes about by means of a free allantois. The thickened entoderm in this hinder part of the ring is especially marked in *Manis*. After a comparatively short time the annular entodermal region has ceased to be a focus of mesenchyme production; henceforth the increase of the vasifactive tissue is left to mitoses of the cells already constituting it. We may after careful consideration of all the mammalian preparations at our disposal all the more safely conclude to the existence of such migration of vasifactive cells if we consider that in other vertebrates (*Teleosts*) this very phenomenon has been actually observed in the live embryo by Wenckebach ('86), Ziegler ('87), and others.

In how far yet other tissue than blood-vessels and blood pathies with Kleinenberg's ('86) drastic expression, "*Es giebt kein mittleres Keimblatt.*"

spring from this entodermal proliferation will require very close investigation in all the different orders of Mammals.

2. Developmental Processes in the Ectoderm.

I have on purpose postponed the discussion of the processes of proliferation in the ectoderm because in modern textbooks those in the entoderm are generally ignored or even denied, whereas as a matter of fact they are antecedent, at least in their very earliest appearance, to those which concern the ectoderm.

About the latter a very stately list of investigators, including many of the foremost embryologists, have published the results both of observation and of reflexion. Still we cannot say that at present a general consensus of opinion concerning these processes has been arrived at. They have been very ably summarised by O. Hertwig in his "*Lehre von den Keimblättern*" ('03, pp. 918—940), and to that author I would direct those who are interested in the historical development of the different views held on this point.

This will give me occasion to skip at the present moment all controversial matter, and will allow me to put forward my own view of the case based on the examination of numerous early stages of different mammals. The point of divergence with other authors will then be noticed only afterwards.

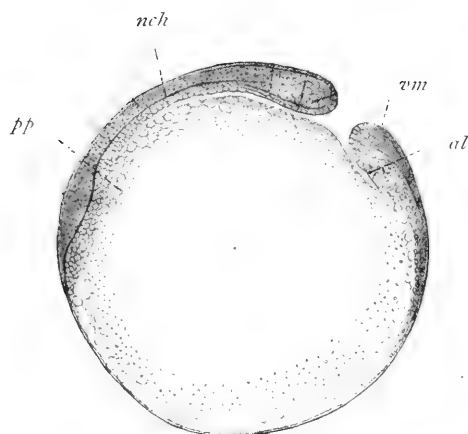
a. The Protochordal Wedge.—At the time when the two germ-layers of the round or oval embryonic shield are not yet interlocked, but independent of each other, and when the future front region of that shield can already be distinguished by the proliferation in the endoderm noticed in the preceding paragraph, and many years ago designated by me ('90) by the name of protochordal plate, a proliferation, directed downwards, of the ectoderm in the axis of the embryonic shield and somewhere in the posterior third of it, becomes visible. I have no hesitation in saying that this spot coincides with the anterior lip of what was described in Chapter II as the

evanescent blastopore of the didermic gastrula stage of mammals. However, only in some few mammals has this blastopore been shown to appear as an actual though very temporary and evanescent perforation of the embryonic shield (Figs. 53—57). The proliferation has been known by the name of its first observer as "Hensen's knob;" it has also been called the primitive knob (Bonnet, '89, pp. 38 and 40); for myself I wish to adhere to the name I have proposed for it many years ago ('90, p. 501) and call it "protochordal wedge," as I have called the entodermal proliferation "protochordal plate." The point of importance in my wishing to stick to these names is that the next step in mammalian development is the firm fusion between these two independent proliferations that have arisen in quick succession in the two independent germ-layers, and that will henceforth be no more disconnected (Figs. 47, 48, 52, 57, 97—99). The notochord is built up of material situated in the axial line of these proliferations, hence the names.

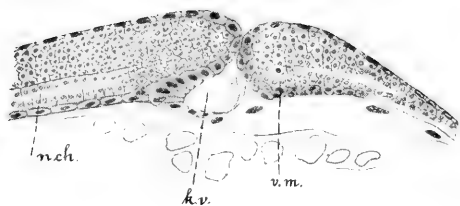
Already Hensen has correctly observed ('76) that below the rounded knob which he found projecting downwards from the ectoderm, the degree of firmness with which entoderm and ectoderm cling together is at its maximum, and should be looked upon as an effective fusion of the two layers. This is fully confirmed both by transverse and longitudinal sections. I found the same in the shrew (Fig. 57) and more lately, in a yet higher degree, in *Tarsius* (Figs. 48 and 52).

In *Tarsius* where we have already seen on p. 32 how very massive the protochordal plate was, the protochordal wedge pushes downwards just behind it, over that part of the entoderm which again consists of flattened cells. The fusion between the proliferated endoderm and ectoderm cells, not yet effected in the section of Fig. 49, comes about immediately afterwards (Fig. 48). There is not the slightest evidence in *Tarsius* that the knob-like ectodermal proliferation which we have called the protochordal wedge undergoes any extension forwards which could be identified with what German authors

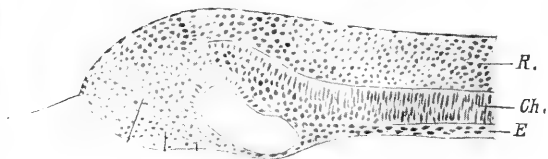
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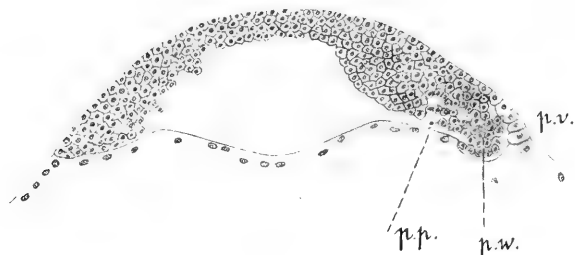
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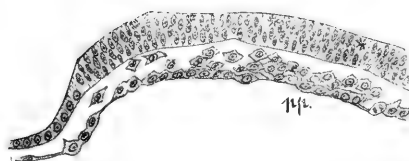
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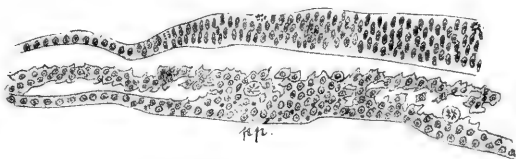


Fig. 87. Longitudinal section through an early embryo of *Amia* (after Bashford Dean, '96). This stage is comparable to that of *Rana* (Fig. 80) and of *Hypogeophis* (Fig. 85); *pp* protochordal plate, *nch* notochord, *vm* ventral mesoblast, *al* portion of intestine comparable with allantois region of higher vertebrates. — Fig. 88. Longitudinal section of early muraenoid embryo (after Boeke, '03). *pp* protochordal plate, *pv* protochordal wedge, *pv* proliferation homologous to the ventral mesoblast. — Fig. 89. A section of the hinder portion of an older muraenoid blastodisk. *vm* ventral mesoblast. *kv* Kupffer's vesicle; *nch* notochord (after Boeke, '03). — Fig. 90. Longitudinal section of the hinder part of a Salmonid embryo with Kupffer's vesicle (after Ziegler, '02). — Fig. 91 and 92. Two sections through the anterior part of two different blastodisks of the dog (after Bonnet, '01). The protochordal plate (*pp*) is proliferating in both of them.



have called the "Kopffortsatz." On the contrary, the moment the fusion with the protochordal plate has come about, a process of growth sets in of the tissues here considered, not in a proximal, but in a distal direction. As a comparison of Figs. 48, 52, 98, and 99 shows, the embryonic shield increases in length, and at the same time the distance between the spot where the protochordal wedge has originated and the front end of the ectodermal shield becomes more considerable. But during this process the situation of the point of fusion between protochordal plate and protochordal wedge may be said to be more or less constant (though not actually any longer discernible), whereas both plate and wedge have increased in length at a relatively equal rate (see Figs. 93—95). And so the protochordal wedge becomes undoubtedly lengthened, not, however, by its sending out any "Fortsatz," but by its being, so to say, "spun out" in consequence of the backward growth of the tissue that is going to be the notochord,¹ thanks to new ectodermal proliferation being added to what had previously come into existence, and had fused with the endodermal protochordal plate. A thin canal is noted in mammals in the posterior part of the backward proliferation of this protochordal wedge (Figs. 98 and 99, *nc*, *cn*).

b. The Ventral Mesoblast.—We will now for a moment leave the protochordal wedge and inquire whether, besides this, any further contribution of the shield ectoderm towards the formation of tissues between it and the endoderm takes place.

In this respect *Tarsius* has proved to be a genus of mammals, which is of the utmost importance in throwing light upon these much disputed questions. Monkeys and man—

¹ I am inclined to think that, if all those investigators that have stood up so decisively for a forward growth of the mammalian "Kopffortsatz" in other genera of mammals, were once more to look closely at their preparations, they would be willing to leave the possibility open that this forward growth may also in their case be an elongation, by material being added posteriorly, concomitantly with the increase in length of the shield.

as soon as we come to know their development in these same early stages—will, in all probability, fully confirm what Tarsius teaches us, considering that in so many other important points Tarsius is seen to resemble the other Primates most closely, and that in this very detail: the presence of an extra embryonic cœlom at a stage ever so much earlier than in any other mammal, there exists perfect uniformity.

In Tarsius there is no doubt that before the appearance of the protochordal wedge (Hensen's knob) in the posterior third of the ectodermal shield, another ectodermal proliferation has already preceded Figs. 47—50, *vm.*), the products of which have important parts to play in the constitution both of embryo and blastocyst, different, however, from those of the protochordal wedge.

This earlier ectodermal proliferation is primarily directed backwards (Fig. 49), whereas the protochordal wedge has a faint inclination forwards (Figs. 46 and 48). Like this it is median and unpaired.

We will call this posterior proliferation the origin of the ventral mesoblast (Hubrecht, '02, pp. 19 and 31), and we may emphasise that, whereas the wedge appeared in the hinder third of the ectodermal shield, this ventral mesoblast originates still further back (separated from the wedge by the potential blastopore) at the posterior extremity of the embryonic shield, there where the trophoblast is often quite sharply differentiated (cf. Figs. 48 and 49) from the embryonic ectoderm. We encounter this proliferation as soon as the entoderm after its delamination from the embryonic knob is busy forming a vesicle under the embryonic ectoderm (Figs. 44 and 62). This endodermal vesicle, as was seen in the preceding chapter (p. 8), never fills the whole of the blastocyst. Now the proliferation at the hind end of the embryonic ectoderm, which we consider as the primordium of the ventral mesoblast, hollows out at the very earliest moment, thus forming a second vesicle enclosed inside the trophoblast. The cavity of this vesicle should be classed as extra-embryonic cœlom; its walls, where applied against the trophoblast

94

96

93

nc

nc

96c

96a

96b

amn

amn

all

st

Trw

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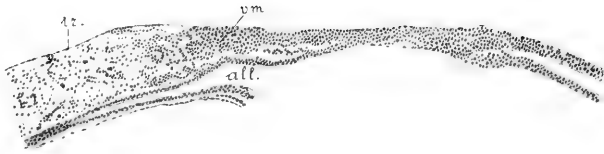
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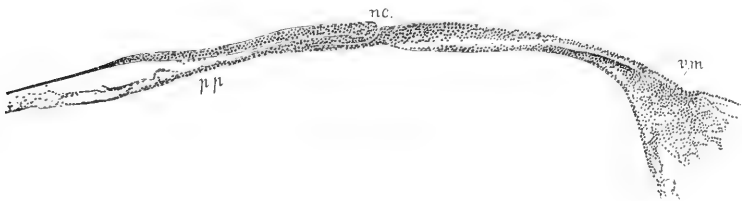
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Fig. 93 to 96. Four surface-views of the embryonic shield of *Tarsius*. In Fig. 93 the median concrescence of protochordal wedge and protochordal plate has come about and notogenesis has commenced; in Fig. 94 and 95 the region of the dorsal mouth (primitive streak) has become elongated simultaneously with the

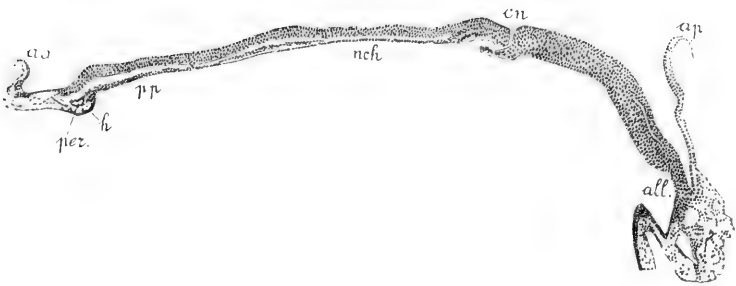
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early establishment of notochord and bilateral mesoblast; in Fig. 95 first indication of neurenteric pore which in Fig. 96 has travelled backwards considerably. — Fig. 96a, b and 96c. Two further stages of Tarsius development following upon those of Fig. 93—96. — Fig. 96a and 96b. Dorsal and ventral view of a blastodisk with about five somites. *As* Headfold seen from below; *amn* amnionfold; *all* allantoidean tube, seen by transparency; *allM* wide mouth of allantoidean tube in umbilical vesicle; *nc* neurenteric pore. — Fig. 96c. Later dorsal view, amnion nearly closed. *st* connective stalk, *trw* part of trophoblastic wall of blastocyst. — Fig. 97, 98 and 99. Longitudinal sections of the embryonic shields respectively corresponding to Fig. 94 to 96. *tr* trophoblast, *vm* ventral mesoblast, *all* allantoic tube (present, but not indicated in Fig. 98). *pp* protochordal plate; *nch* notochord; *cn*, or *nc* neurenteric canal; *aa* and *ap* anterior and posterior amnionfold; *per* pericardium; *h* heart. Fig. 93 to 99 after Hubrecht (02).

(which then becomes a diplotrophoblast or chorion) thereby render the peripheral blastocyst didermic, and may be styled parietal or somatic mesoblast; where applied against the endodermal vesicle they fall under the category of visceral or splanchnic mesoblast (cf. Figs. 45 and 63).

At the initial spot from whence the proliferation has started the ventral mesoblast is naturally more massive than in the peripheral, flattened portions, and may here be designated as the material out of which the primitive streak and the ventral stalk (Haftstiel, Bauchstiel) of the *Tarsius* embryo takes its origin. This stalk-shaped connection between embryo and trophoblast is thus present in the very earliest stages of development (Fig. 62).

My conception of the ventral mesoblast in mammals has since been adopted by Rückert in his article above cited ('06, pp. 1248 and 1251). He compares it with the observations hitherto recorded of mesoblast formation in the same region in other Amniotes. From its posterior unpaired and median point of origin in *Tarsius* it gradually spreads forward right and left as the wings of mesoblast are known to do in other mammals ("Mesoderm-sichel"), and only later this vesicular mesoblast (vesicular, because the coelom is there from the beginning, and does not, as far as the extra embryonic coelom is concerned, originate by any ulterior splitting process) also appears in front of the embryonic shield, and invades the space (cf. Figs. 62 and 63) where the anterior and superior entodermal surface of the umbilical sac are yet in close opposition with the trophoblast (Hubrecht, '02, Figs. 48, 51c, as compared to 57a, c). The posterior median portion has simultaneously further developed into the incipient, as yet extremely delicate Haftstiel which as we saw is there from the very beginning, i. e. from the didermic stage downwards.

3. Mutual Relations between the Centres of Proliferation.

We must now consider the relation in which on the embryonic shield the centre of proliferation of the ventral mesoblast stands to that, which we have designated as the protochordal wedge. In general it may be said that in the earlier stages the former lies immediately behind the latter. We may add to this that if *Tarsius* were possessed of a blastopore in the didermic gastrula in the same way as *Erinaceus* is (and as are some other mammals) the situation of this blastopore would be such as to separate these two centres of proliferation. This becomes evident when we consider the exceptional case already noted above (p. 14) where the embryonic shield of a particular specimen of *Tarsius* was provided with a deep pit-like impression (Fig. 52) which cannot but be looked upon as an attempt at blastoporic perforation of atavistic significance, the very numerous stages of *Tarsius* of identical age which I have in my possession not revealing a trace of it.

Other cases in which the contiguity, but at the same time the mutual independence of the two centres of proliferation is evident were figured by me in a former publication ('02, figs. 58*b*, 46*d*, 47, 48, 52, *b* and *c*).¹ Out of them all (see also Figs. 47, 48, 49, and 50) I have constructed the semischematic diagrams, Figs. 44—46.

I need hardly explain that the presence, close to each other, of three centres of proliferation (one entodermal, two ectodermal) in the two germ-layers of the mammals, such as we have just described, combined with the fact that in each centre new cells are very actively developed which spread out in the only direction available to them, i. e. between these two germ-layers,

¹ I will here notice that the mutual independence here insisted upon should be taken *cum grano salis*. The anterior and the posterior lip of the blastopore, being naturally connected by the lateral lips, it is not a material anatomical independence that is here meant, but an independent activity. On p. 44 this will be more fully entered into.

brings about a state of things in which it very soon becomes utterly impossible to say to which of the three centres a given cell or group of cells owes its origin. An intimate fusion, though withdrawing this question of cell-lineage out of the field of our powers of discrimination, does not, however, diminish the significance of the existence of such a cell-lineage,¹ and we will in future researches have to keep our attention directed to that point, even though we must recognise at the present moment that much of the confusion and of the erroneous notions that maintain such a hazy atmosphere round these important early phases of vertebrate development, is due to precocious generalisations on this head. It seems to me that the wish to uphold the reality of a third germinal layer, together with the ardent desire of not having to ascribe a multiple origin to it, is responsible for much theoretical dogmatism that will henceforth prove valueless.

The consequence of what we have here described for *Tarsius* is that the centres of proliferation which give rise to the protochordal wedge and to the ventral mesoblast are originally independent of each other. We shall by-and-bye see that there is all reason to believe that the same holds good for all other Mammalia, aye, for all other Vertebrates. The principal difference between my own and the current views consists in the distinction which I wish to make between what was considered as the front portion of the primitive streak (Hensen's knob, of which even the anterior prolongation was called in full: "*Kopffortsatz des Primitivstreifens*") from the primitive streak material itself. This distinction, which is very soon effaced and could never be demonstrated in later stages, is, however, quite evident in the very early ones. And we will have to analyse, as acutely as we can, the differences this will call forth in our interpretation of the development of different tissues and organs concerned.

The ventral mesoblast at its very earliest appearance (also in *Tarsius*) may be said—as it springs from the hinder end of the ectodermal shield—to be more or less crescent- or fan-

¹ Vide E. B. Wilson ('92, '97), as against Driesch and others.

shaped. We will again encounter this crescent (or "Sichel") shape in Sauropsids. But as the embryonic shield increases in length the centre of proliferation is equally stretched, and out of a crescent shape evolves a double wing-shape, the axis between the two symmetrical wings being in the axis of the embryo. Along this axis the ectoderm freely produces cell material penetrating downwards to the right and to the left between the germinal layers and forming what has often been designated as primitive streak-mesoblast continuing backwards in the median line as the connective stalk (Haftstiel).

Here we encounter an all-important phenomenon, which will be better understood when we have also considered it phylogenetically, and which consists in the substitution of what was at the outset the blastopore by what has later developed into the dorsal mouth-slit. The lengthening of the tissue which formed the lateral lips of the early blastopore has now set in, and the further proliferation of this tissue, concomitant with a process of coalescence of the right and left halves with reminiscences of the original lumen which was the slit-like cavity of the stomodæum (in the cœlenterate stage), brings our original centres of proliferation further apart. At the same time the continuity of the tissues is never interrupted.

The accumulation of cell material which represents the lateral lips of the dorsal mouth-slit (Rückenmund) naturally causes an increase in length of the mammalian embryonic shield, during which the shape of the shield generally changes from a roundish to an oval or pear-shaped one (see Figs. 93—95). This lengthening is simultaneous with an increase in the extension of the lateral mesoblast wings (see Fig. 60). For *Tarsius* I have fully established this a few years ago ('02, Figs. 54, 57, 61, 72). And for other mammals it has been demonstrated by Bonnet ('97, Figs. 18, 19), Keibel ('93, '95), and others.

As soon as this accumulation of material that reveals itself in the increase in length of the embryonic shield has

reached a certain stage, an active process of transformation is inaugurated, which consists in the visible differentiation of all-important organs, notochord and somites, out of this matrix. The differentiation becomes first visible at the front end where our ectodermal proliferation, the protochordal wedge, has grown downwards and has coalesced with the protochordal plate. From this point backwards the notochord is now, so to say, spun out, the so-called primitive streak tissues—lateral lips of the dorsal mouth—at the same time diminishing in extent. Phylogenetically it corresponds to the origin and the coalescence of the lateral lips, not of the blastopore, but of the dorsally-situated stomodæum.

A comparison between Figs. 93—95 and 96 will at a glance reveal the effect of the new state of things. The protochordal wedge situated well forwards on the embryonic shield of Fig. 95 is no longer visible as such. The fine pore that was present just behind it (see the longitudinal section in Fig. 98) has undergone a displacement backwards, and has in Fig. 96 attained a position not far from the hinder end of the embryonic shield. This is due to a very marked process of elongation which becomes perfectly evident on the comparison of two longitudinal sections through these two embryonic shields (Figs. 98 and 99).

This process has been known to earlier observers, and has been described as the shortening of the primitive streak, going parallel to the formation of the earliest somites. How the cell material that has arisen as the paired wings of the ventral mesoblast and that which is spun out by the moving backwards of the protochordal wedge (producing the notochord in the median axis and the mesoblastic somites right and left of it) comes to arrange itself reciprocally and what changes are brought about in this material during this process is a very difficult and intricate question about which the various authors differ. I think we may safely say that by the rapid extension backwards of the differentiation process, as it is exemplified by Figs. 95 and 96, the dorsal region of the trunk is laid down in outlines (hence the word

notogenesis), whereas the derivatives of the ventral mesoblast find employment in the construction of the posterior and postero-ventral portions of the embryo.

It will here suffice to state that the extra embryonic coelom which is present in *Tarsius* (and undoubtedly in monkeys and man) in the ventral mesoblast at so very early a phase (extending as was described on p. 38 behind and below the endodermic vesicle and the ectodermal shield) first makes its appearance in the other mammals at a later period, but exactly in the same position, viz. behind the embryonic shield (Figs. 43, 61, and 100). From there it gradually extends in crescent shape right and left along the hind margin of the embryonic shield. This coelom—considerably less spacious and less precocious than that of the Primates—is fully homologous to it, both as regards the place where it is found, the cell material in which it appears, and the relation in which it stands to the coelom of the somites and lateral plates, as will be described later on. Bonnet's ('82, '89), Keibel's ('93), and my own ('02) observations on the appearance of this crescent-shaped coelom are in perfect agreement with each other, as also those concerning the fact that this ventral coelom only later fuses with the intra-embryonic coelom (Keibel, '93, figs. 39 and 40*x*; Hubrecht, '02, fig. 77*d*). The pericardial coelom arises independently along the front border of the embryonic shield, and will also be more fully discussed later on (Hubrecht, '02, p. 37, figs. 70, 73).

Summarising what we have here rapidly sketched we may agree to have seen that instead of a homogeneous median germ layer, instead of a mesoderm which has the same morphological importance as the two primary germ layers and originates from the coalescing lips of a blastopore, we find at least three foci of cell-activity in those primary germ layers. The appearance of these foci marks the end of the didermic stage of the blastocyst. In consequence of processes of proliferation and rapid mitosis there is started from these three centres a host of new cells, which, together, spread between



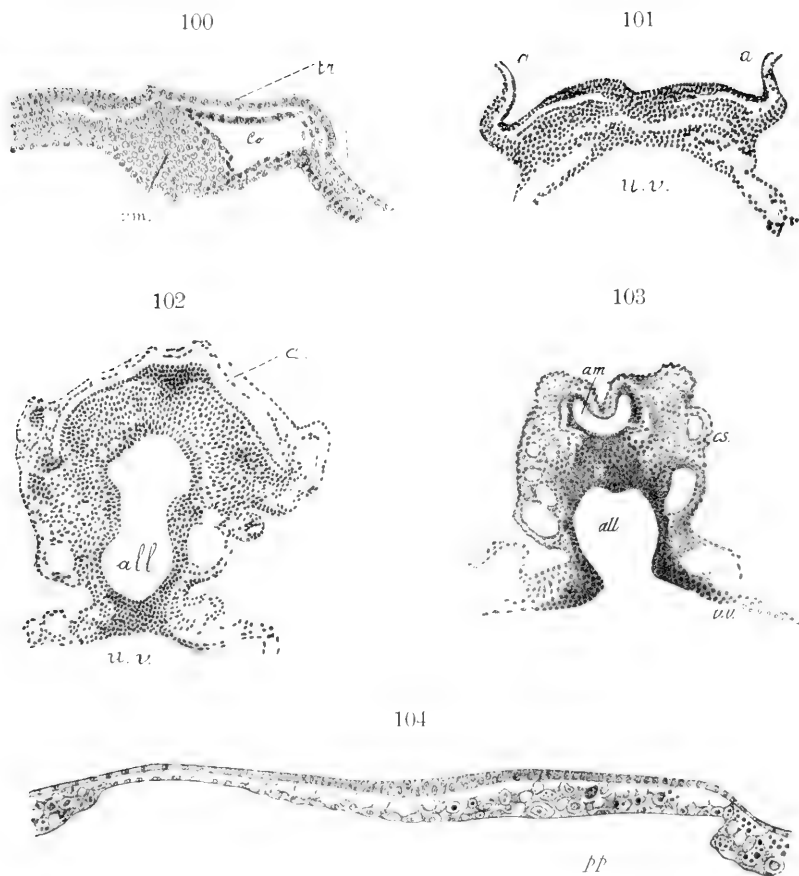


Fig. 100. Longitudinal section through the posterior end of the shrew's blastodisk with earliest appearance of posterior amnionfold (after Hubrecht, '90). *co* posterior coelom, *vm* ventral mesoblast, *tr* trophoblast (cf. Fig. 56 and 61). — Fig. 101, 102. Two sections through different blastodisks of *Tarsius* (nos 675 and 180 Utr. coll.) in the posterior region of the primitive streak (after Hubrecht, '02). Fig. 102 is in the more posteriorly situated, at the spot where the tailgut (Schwanzdarm) and the allantois *all*, here situated ventrally of the former and on the point of diverging. The wall of both is actively proliferating vascular tissue. Between the lower border of the allantois and the umbilical vesicle a complex of yet more actively proliferating cells is present: this continues further backward (where allantois and umbilical vesicle have become further severed) as a median proliferating raphe on the umbilical vesicle. In Fig. 101 lateral wings of mesoblast arise from the entoderm. — Fig. 103. Transverse section through the hinder end of an early *Tarsius* embryo with tubular amnion (*am*) and allantois (*all*) in the already strongly vascularized connective stalk *cs*; *uv* wall of the umbilical vesicle. — Fig. 104. Longitudinal section of sparrow with early mesoblast in a stage of about Fig. 105 (after Schauinsland, '03).

ectoderm and entoderm in the shape of what appears naturally as a flattened layer of so-called mesoderm, but what is in reality the strictly grouped material for different organs and tissues. These have not sprung from the lips of any blastopore (Urmund), but have gradually come into existence in the same ontogenetical order as we must expect them to have arisen phylogenetically. The blastopore has lengthened out into the dorsal mouth. This lengthening has been accompanied by a dorso-ventral proliferation of ectoderm (protochordal wedge) out of which the stomodæum (notochord) arises, and during this time the dorsal mouth-slit has only been represented by vestiges. I have already discussed these processes elsewhere ('05). The dorsal, elongated mouth (Rückenmund, '05, p. 363) may thus point to a vermactinian-like ancestor (Fig. 160) in which the appearance of notochord and cœlomic pouches was already foreshadowed by the stomodæum and the enteric diverticula to which the stomodæum gives access.

It is far outside the scope of this paper to establish in detail the cell-lineages as they may ultimately be found to exist, and which will some day allow us to ascribe to each of the three centres of proliferation here alluded to, its part in the formation of the Anlage of different organs and tissues between ecto- and entoderm. It should, however, be noticed that already in my publication of six years ago ('02, Pls. 8 and 9, figs. 59*g* and 75*h*) I have distinctly figured the fact that in the posterior region of the embryonic shield a very considerable part is played by the entoderm in the development of the lower half of wings of mesoblast of which the upper half springs directly from the ectoderm (Figs. 101—103).

These and many other phenomena will have to be minutely studied and established before we can commence our comparative analysis of these processes in Vertebrates.

But it should be borne in mind that the processes just alluded to have already been mentioned on p. 34, when the vascularisation of the "Haftstiel" was discussed. And that in the diagram, Fig. 46, the posterior source of proliferating

entoderm is clearly indicated as forming part of the ring that is figured for *Sorex* in Fig. 60.

The ultimate discussion upon this matter is postponed to a later publication, in which those stages of development which are inaugurated by the formation of the somites will be treated more fully.

II. AMPHIBIA.

After this description of the early developmental processes of the Mammalia we will skip the Sauropsida, and first describe what is noticed in the Amphibia. This will afterwards afford us an occasion to compare the yolk-laden Sauropsida all the more rapidly both ways. And, above all, it will increase our confidence in the interpretation which we have founded on the Mammalia if we find it applicable as low down in the line of vertebrate descent as are the present Amphibia. It should, however, at the same time, be remembered that none of the three living stems of Amphibia neither the Gymnophiones, nor the Urodeles, nor the Anura can be expected to stand in any way on the direct line of descent of our present mammals. Comparative anatomy has taught us (Fürbringer '00) that in very many respects the amphibious Promammalia of the Palæozoic epoch must have been characterised by important points of difference from all the living remnants of that ancient stem. Still, if we find processes of early development that are in the main lines directly comparable to what we have described in mammals, and if they fit in well with the explanation which we have ventured to give for the Mammalia, we might say that the difficulties which have so often been complained of (p. 13) when attempting to establish the comparative ontogeny of the Vertebrates have greatly diminished.

We will, therefore, take the more important and careful descriptions of Amphibian development (to which we have no personal investigations of our own to add), and see whether the three centres of proliferation which we have noticed in

the two germ-layers of the mammals are also present in the Amphibia, and whether the mutual relations of these proliferating centres and the further fate of the tissues and organs they produce also reveal close similarity.

We begin with the Gymnophiones, about the early phases of which A. Brauer ('97) has published an exceedingly lucid exposition based on the study of an extensive material.

We have successively to look out for a proliferation of the entoderm corresponding to our protochordal plate, for an ectodermal proliferation representing the protochordal wedge, and for another ectodermal centre of growth which gives rise to ventral mesoblast. I will by means of copies of Brauer's figures show that all three are met with in *Hypogeophis* and that in their further relations and in the genesis of the organs produced by them the homology with the mammals is indubitable.

It should at the outset be remembered that the *Hypogeophis* egg is so saturated with yolk-material, that there is no holoblastic cleavage, but that the results of the cleavage process—as was noted in Chapter II—are found at one pole of the egg, and that a process of delamination transforms the fragmented ovum without delay into a gastrula with an entodermic lower layer (see Brauer, '97, Figs. A and B, pp. 403, 404).

More or less simultaneously a differentiation of ectoderm and endoderm becomes visible, which shows very great similarity with what was figured above (Fig. 19) for *Tarsius*. The point at which the ectoderm has commenced to proliferate, and at which its first change was a bend downwards (Fig. 83) is directly comparable to the primitive (Hensen's) knob on the mammalian ectodermal shield, and is no other than our protochordal wedge. The point at which the endodermal proliferation becomes evident is situated just in front of it and the two proliferations, as is so particularly clear in Brauer's fig. 43, here reproduced as Fig. 84, fuse in absolutely the same way as we see in the *Tarsius*, Fig. 48; with full confidence I indicate the corresponding regions in the Amphibian

by the letters *Pp.* (protochordal plate) and *Pw.* (protochordal wedge). Brauer's figure leaves not the least doubt that the cells indicated by *Pp.* are of entodermal, and the cells *Pw.* of ectodermal origin, nor do his own views on this point differ from mine as he calls the former "vegetative," the latter "animale Zellen."

The later transformation of this henceforth fused region of double proliferation (see Figs. 85 and 86), fused on entirely the same plan as was noted not only in *Tarsius* but in very numerous other mammals, will be discussed later on. We must first look out for the third centre of proliferation. And we find this in Brauer's fig. 59, here copied in Fig. 86 where at a short distance behind the protochordal wedge and separated from it by an interval comparable to what we notice in the *Tarsius*, Figs. 46 and 48 (where the interval is at its minimum), the ectoderm is seen to undergo a new and very marked proliferation, which will give rise to tissues closely corresponding to the ventral mesoblast which we saw originating in this spot in mammals.

The difference between the case of *Tarsius* and *Hypogeophis* is this, that in the former this posterior centre of proliferation is conspicuous first of all, whereas in the latter the two other centres have precedence. However, in this respect the other mammals side with the *Amphibia*, the protochordal plate and wedge being visible before or arising simultaneously with the proliferating centre for the ventral mesoblast.

Having thus established well-founded comparisons between Brauer's figures of early *Gymnophiones* (*Cœcilia*) and our own for mammals, we will now turn to the *Anura*, and take as starting-point Brachet's figures ('03) of the frog.

In his earlier publication of the year 1903 (Figs. 6, 7, 39—47) we find that Brachet describes early stages both for the *Axolotl* and for the frog in which the presence of a protochordal plate can hardly be denied by any impartial observer. One of his figures, copied here in Fig. 79, leaves little doubt about the presence in the entoderm of the frog of a

particular spot of thickened entoderm situated at the very place where we would expect the protochordal plate at this earlier stage. His other figures, which are also copied here (Figs. 80—82) show the subsequent stages.

And as to the annular band of entoderm from which the blood-vessels and blood originate, we find it similarly disposed in the Amphibia according to Brachet, who for the frog writes ('03, p. 686), "*les endotheliums vasculaires y compris l'endothelium endocardiaque et les futures cellules rouges du sang, procèdent de la portion du mésoblaste . . . qui s'est séparée par délamination de la partie ventrale de l'endoblaste gastruléen.*" And further ('03, p. 688), "*de tout le vaste manchon mésoblastique qui se délamine à la surface de l'endoblaste gastruléen, la partie ventrale, sur une largeur plus ou moins grande selon les régions, se sépare complètement du reste à des stades relativement peu avancés, et, poursuivant dès lors une évolution spéciale, donne naissance à tout l'appareil vasculaire sanguin (endotheliums vasculaires et cellules rouges du sang).*"

The term *manchon* (muff) used by Brachet shows that he, too, has observed the region of the entoderm which produces the mesenchyme out of which the blood-vessels and the blood are developed in the shape of an annular peripheral investment of the region out of which the mediodorsal organs will develop.

With most laudable prudence Brachet does not generalise his results concerning the frog, but states expressly that for Triton he is inclined to stick to the conclusion he arrived at already in an earlier publication ('98), and according to which also in Triton the vascular system is of entodermic origin, but that he all the same thinks a further confirmation of his observations desirable, whereas for Axolotl he makes all reserves, the study of the origin of the vascular cells being here very difficult. He is careful to add, however, that he cannot exclude the possibility that after all Axolotl may prove to fit into the same plan as the two others.

Other authors who, before Brachet, have come to similar

conclusions concerning the origin of the vascular system in the Amphibia are Goette ('75) and Schwink ('91). Both are convinced that all blood-cells are derived from the endoderm, as also the vessels. Brachet points out, however, that the stages on which Schwink bases his conclusions are already too far advanced.

It is important that Brachet, repeating Corning's ('99) observations, finds that in front of the notochord's anterior end the median protochordal-plate-material differentiates from behind forwards in this sense that mesoblast is seen to become isolated and to form a thin layer made up out of one or two layers of cells that are interposed between the endoderm and the lower brain wall. In the beginning he finds that the anterior end of the notochord reaches into this median mesoblastic band. . Soon, however, it is separated out of it and the anterior extremity of the notochord becomes quite free. Later yet the median mesoblastic band thins out, breaks up and ultimately disappears, or is reduced to a few sparse cells that are distributed at random. The endoderm of the roof of the digestive tube is then closely pressed against the lower wall of the brain.

This would, *ceteris paribus*, also apply to the mammalia.

The next point we have to consider concerns the fusion described for mammalia by myself ('90) and others, and for *Hypogeophis* by Brauer of what we have called the protochordal plate with the protochordal wedge. Neither amongst Brachet's figures for *Axolotl*, nor among those for the frog, are the phenomena so self-evident as they were for Brauer's *Hypogeophis*. Still if we consider Brachet's figures of *Axolotl* and those for the frog no objection can reasonably be raised against my comparing the region which in all these figures I indicate by *Pp.* with that same region in *Hypogeophis* and in mammals. The fusion with the ectoblastic proliferation that is the protochordal wedge—although Brachet does not look upon it in that light—is inaugurated for *Axolotl* in Brachet's ('03) Figs. 4 and 5; for *Rana* in Fig. 79, here given. The ectodermal proliferation indicated as protochordal wedge is

thus located in the Amphibia (the same holds good for Hypogeophis) at the spot where the so-called dorsal lip of the blastopore makes its first appearance. And this proliferating spot (as was already noticed in Mammals and as Brachet ['02, '03], Bellonci ['84], and Lwoff ['94] have observed it for Amphibia), travels backwards a certain distance over the surface of the egg, spinning out at the same time both notochord and somites.

In the frog I have in the reproduction of Brachet's figures indicated the corresponding regions by the letters *Pp* and *Pw*. *Pp* points to most decided entoderm which has by delamination become separated from the ectoderm situated above it. And the proliferation of ectoderm marked *Pw* and fused (more markedly yet than in the somewhat earlier phase of Axolotl) at the very outset of its proliferation with the protochordal plate entoderm below it—about in the same way as we encounter the same phenomenon in Fig. 48 for Tarsius—has here already progressed a certain distance backwards. This distance has lengthened and the derivatives of what was originally the protochordal wedge have increased in Fig. 80 (*Rana*), and yet the letters *Pp* leave no doubt but that they are directed towards the original entodermal proliferation. So does *Pw* show us what has become of the ectodermal proliferating centre. We can quite understand that also concerning Amphibia dissensions have existed as to whether the notochord—first and foremost derivative of *Pw*—was of entodermal, mesodermal or ectodermal origin. And the different authors that have successively sided for the one or for the other of these solutions have pronounced themselves as best they could upon a material that is so far away from the extraordinary clearness with which these very early phenomena reveal themselves to us in Mammals. The continuity in which from the beginning ectoderm and entoderm pass into each other (Fig. 79) all along the ring-shaped zone of delamination (marginal zone of Goette), has contributed so extraordinarily to propagate and to strengthen the error of those who upheld that the phenomena which we have just seen

inaugurated are phenomena of gastrulation, instead of phenomena of notogenesis, that consequently all conclusions were naturally biassed. However, the mammalia have come to show us the way out of the labyrinth and a reformation of our views must be the consequence.

Without for the present entering into further details concerning protochordal plate, protochordal wedge and their derivatives in the frog, we will now see whether the third centre of proliferation which we also have recognised in Brauer's *Gymnophiones* is as clear in the *Urodeles* and the *Anura*.

On this point Brachet's researches, and those of other authors afterwards to be cited, leave no doubt whatever. The ectodermal centre of proliferation, hitherto known as the ventral lip of the blastopore is very clearly marked off (Figs. 80—82), and produces its mesoblastic derivatives with perfect regularity, in a sequence that is immediately comparable to what we have found in and described for the mammals. Brachet writes about this third centre ('03, p. 67) that it is: "Un épaississement notable de la partie toute inférieure de l'ectoblaste." And further (l. c., p. 68): "Même épaississement considérable de l'ectoblaste qui vient par une large base se continuer avec les éléments du bouchon vitellin et cela à une certaine distance dans la profondeur de l'œuf."

We have here before us the ventral mesoblast which in *Tarsius* (and the other *Primates*) arises so uncommonly early and stretches round the umbilical vesicle, creating a very early segregation of splanchnic as against somatic mesoblast.

When we take the Figs. 81 and 82 (copied from Brachet) we can immediately recognise the homology between the region marked *vm* and that of Figs. 46, 48, and 49, to which the same lettering is attached. We also see that if the mesoblast there produced in the *Amphibia* were to attain the early development it has in *Tarsius* and the cavity enclosed therein, this would similarly take the place of the so-called segmentation cavity, and be applied against the

cavity which in the mammal is styled the umbilical vesicle, and which is the so-called archenteron in the Amphibian. The concrescence between this and the segmentation cavity is the same as is noticed at the yet earlier stage of *Tarsius* (Fig. 19), in which the entoderm forms the roof of the trophoblastic cavity. But we will return to these possible comparisons later on.

It remains to be seen whether in other Amphibia than *Rana* and *Triton* the presence of a fourth focus from whence tissues are originated that take their place between ecto- and ento-derm can be confirmed. In other words, whether anything corresponding to the annular zone of mesenchyme-producing entoderm (stretching backwards right and left from the protochordal plate and reuniting in the median line posteriorly under the ventral mesoblast) as it was figured in Fig. 60 occurs in Amphibia.

Although Brachet has not expressly stated that such an annular zone of entoderm was noticed by him, we may conclude from his descriptions that it does occur in his preparations. On p. 88 ('03) he writes about: "*L'intense activité que l'on pourrait appeler mésoblastogène des cellules de la voute*" (by which latter he means the roof of the archenteron); and on p. 89: "*Les bandes mésoblastiques sont plus épaisses dans la région blastoporale que dans la région gastrale proprement dite . . . Le mésoblaste péri-stomal est beaucoup plus abondant que le mésoblaste gastral* (p. 90)."

From these citations I think we may conclude that the presence of an annular zone of mesenchyme-producing entoderm in Amphibia will in due time be yet more fully established.

Authors who have actually figured it in the posterior median line of the embryonic Anlage are Robinson and Assheton ('91, Figs. 14—17), where in the median region of the blastopore and behind it we notice an entodermal proliferation producing what the authors call "hypoblastic or inner layer of mesoblast of primitive streak," as against

the "epiblastic or outer layer of mesoblast of primitive streak." This investigation thus authorises a direct comparison of the phenomena figured in Figs. 101 and 102 for *Tarsius*, in which we have quite decisively recognised an epiblastic and a hypoblastic layer of mesoblast of the primitive streak, which is what was noticed in the frog by Robinson and Assheton.

It should yet be added that the annular zone of mesenchyme-producing entoderm may in the frog even commence as an unpaired ventral sheet, which only later becomes paired, and thus more or less annular. Brachet ('03, p. 686) expresses this as follows: "Il existe une phase du développement où les cellules vasculaires des futurs vaisseaux vitellins forment une couche continue impaire et médiane (Fig. 22) et la parité définitive est secondaire."

We have now seen that in the three subdivisions of the Amphibia we notice early processes of ectodermal and entodermal proliferation, which allow of direct comparison with what we have described for the Mammalia. And we may add that the continuity between the derivatives of the protochordal plate and those of the annular zone is in Amphibia established even perhaps earlier yet, whereas the continuity of these latter derivatives with those mesoblastic elements that originated right and left of the median dorsal line is again so very early established, that it cannot be wondered at that the Amphibia have not suggested to previous investigators the relative independence of these different sources from whence cells and tissues that will take their places between the two primary germ-layers arise.

When we will later recapitulate what are the further lines of development of the products of the four proliferations above enumerated, the complete homology between Amphibia and Mammals will become yet more evident.

III. SAUROPSIDA AND ORNITHODELPHIA.

As to this class I have no observations of my own to offer. But we may glean from the researches hitherto published by others the following data which concern the participation of the entoderm in the formation of mesenchyme.

For the sparrow *Schauinsland* ('03) figures both a surface view, a longitudinal and a transverse section which leave no doubt about the presence in this bird of a very clearly defined protochordal plate, arising as a proliferation in the entoderm before any process of mesoblast formation has been inaugurated in the ectoderm. I here copy five of his figures (Figs. 105—109), adding that the region in the surface view which I have termed the protochordal plate is named by *Schauinsland* the "Entoblasthof." Not only is its situation in perfect correspondence with the same region in *Sorex*, visible in Figs. 59 and 60, but the sections reveal the same constitution (Fig. 104 for the sparrow, Fig. 58 for the shrew), viz. a local thickening of the entoderm. And later when the ventral mesoblast (cf. p. 39) will have begun to make its appearance the surface view of bird and mammal will again be directly comparable, and the independent increase of tissues—intricately interwoven though deriving from different germ layers—undeniable.

Similarly in representatives of the reptiles there is no want of recent illustrations by various authors, showing that the median entodermal proliferation (protochordal plate) is present in early stages. I copy some of *Passer* (Figs. 104, 110), five of *Sphenodon* (Figs. 77, 78, 112—114), and two of *Chamæleo* (Figs. 76, 111), all seven taken from *Schauinsland*. And I may add that *Mitsukuri* ('93), *Mehnert* ('92, '94), and *Davenport* ('96, Pl. VII) have revealed a similar state of things for tortoises, *Strahl* ('82, '84), *Will* ('90), and *Corning* ('99) for lizards, *Voeltzkow* ('93) for crocodiles.

If the presence of a protochordal plate in Sauropsids may thus be looked upon as well established we have to look a

little more closely before affirming that the same can be said of the annular mesenchyme-producing zone in the entoderm. If we consult Mehnert's article in which he discusses the origin and the development of the hæmovasal tissue (area-vasculosa-crescent) in *Emys* and *Struthio* ('96) we will then find that for these two Sauropsids he accepts as the final point of origin of the vascular tissue and the blood the entoderm. But he does more than that. He gives a detailed account which in most points corresponds most exactly with what we have above indicated for the mammalia, of the origin in the hinder part of the "primitive streak" of a decided entodermal proliferation which by many authors has been incorrectly looked upon as ectodermal. I believe that a careful re-examination of their preparations and a comparison of those with the numerous section series of *Tarsius* and *Tupaja*, which are always available for that purpose, may convince even those who have formerly stuck to the purely ectoblastic nature of the primitive streak, that in the lower half of the primitive-streak-tissue a direct and considerable proliferation of entoderm cannot possibly be denied. This proliferating region is, as we have seen in mammals, nothing else but the hinder median portion of the ring of vasifactive tissue, which was above discussed and figured (Figs. 46 and 60), and of which the protochordal plate is the median frontal portion.

In the tortoise, *Emys*, Mehnert ('96) gives detailed descriptions as to how this ring of tissue has in the first place the aspect of lateral outgrowths from the primitive streak; later of crescent-shaped wings, and only finally of a ring. It may be here remembered that also in the embryonic shield of *Tarsius* the first origin of blood and blood-vessels is observed in the hinder part, and that we notice a similar wing-shaped advance in the distribution of the mesenchyme-producing annular zone. At the same time it should be borne in mind that once the primordium of the vascular tissue having arisen out of the entoderm its further development becomes independent of the region of its origin, so

105

106

*pp**pw*

107

108

*pp**nch**vm*

109

*pp**nch**vm*

Fig. 105 to 109. Five surface views of the early aspect of the sparrow's blastodisk (after Schauinsland, 03). In Fig. 105 there is as yet only an entodermal protochordal plate *pp* (cf. longitudinal section of Fig. 104); in Fig. 106 a downwards growing protochordal wedge *pw* begins its fusion with the protochordal plate; in Fig. 107 mesoblast has grown out from the borders of the elongated dorsal mouth; in Fig. 108 the ventral mesoblast *vm* has made its appearance; in Fig. 109 the sickle-shape becomes visible in the posterior mesoblast. *nch* notochord.

that for example the fact that in *Tarsius* after a certain time we find the whole umbilical vesicle thickly covered with blood-vessels (Hubrecht, '02, Fig. 91) does not imply that they have arisen in loco out of the entoderm. They have become spread over this after they had once taken their origin in the annular zone here more particularly alluded to.

I think it may here suffice to give the reference to Mehnert's article in which he establishes the entodermal origin of a ring of vasifactive tissue both for a reptile and for a bird, and not in this place to describe the process more in full. The more so as it is well known how many differences of opinion yet exist on this head between different authors. The amount of difference can also be gathered from Mehnert's paper, who gives a tabular summary of the different opinions held on this point by no less than thirty-six different authors, grouped under the heads of six different possibilities for the origin of blood and blood-vessels.

Having so wide a divergence to choose between, it is only natural that I should feel inclined to side with Mehnert ('96), O. Hertwig ('83, p. 319), Goette ('74, '75), His ('00), and Rückert ('06) in respect to the origin of the vascular system now that different genera of mammals have provided me with perfectly trustworthy sections from which to conclude to the existence of the annular mesenchyme-producing zone of the entoderm. That for mammals, Kölliker, Keibel, Heape, and others have denied the participation of the entoderm here advocated, and have derived the whole vascular system from the mesoblast of the primitive streak has no doubt its explanation in this fact that they must have consulted later stages of development than those in which the entodermic origin is evident. This latter stage is very soon followed by one in which the participation of the entoderm has come to a close, and in which the further development of the vascular system is now going on between the two primary layers in the so-called mesoblast.

All the points here discussed have been sifted and carefully compared by Rückert and Mollier in the chapter which

they have contributed to Hertwig's 'Handbuch of Embryology.' The student of their article will find no difficulty in accepting the generalisation above arrived at, that the entoderm is the mother tissue from whence the vascular tissue and the blood have taken their origin.

Rückert writes concerning Gecko (l. c., p. 1172): "Ich traf auf eine deutliche Ablösung der hier ziemlich dotterreichen ersten Gefässanlagen von dem angrenzenden hohen und ebenfalls dottergefüllten Entoblast . . . Ihre entodermale Entstehung liegt daher klar zu Tage."

Besides the protochordal plate and the annular zone of the entoderm from whence mesenchyme is produced we also find in the Sauropsida the ectodermal centres of mesoblast formation which we have noticed in the mammals.

The protochordal wedge is to some extent more marked than we found it in the Mammalia; it has of late been designated by O. Hertwig ('06) by the name of "Mesoderm-säckchen," and it contains a cavity more spacious than the comparatively thin canal which we have encountered in mammals (Fig. 98). Also in this respect the exceptional case of Fig. 52 should be considered as throwing a side-light on these intricate processes both in mammals and Sauropsids.

The confluence between the earliest ectodermal downgrowth with the protochordal plate has up to now not been specially examined in reptiles. Still we may conclude from the figures here given, which I copy from other authors that it comes about in exactly the same way as we noticed it in *Tarsius* for mammals, and in *Hypogeophis* for Amphibians. Fig. 419, Hertwig ('06), shows us the earliest protochordal wedge in a snake as it fuses with the thickened entoderm; behind the protochordal wedge we notice the ventral mesoblast as a third focus of mesoblast formation. Figs. 427 and 429 are continuations of the same in somewhat later phases, and the correspondence with our Figs. 79, 80, 97, and 98, of Amphibians and Mammals is self-evident. In Hertwig's Fig. 429 the source of ventral mesoblast has actually been shifted

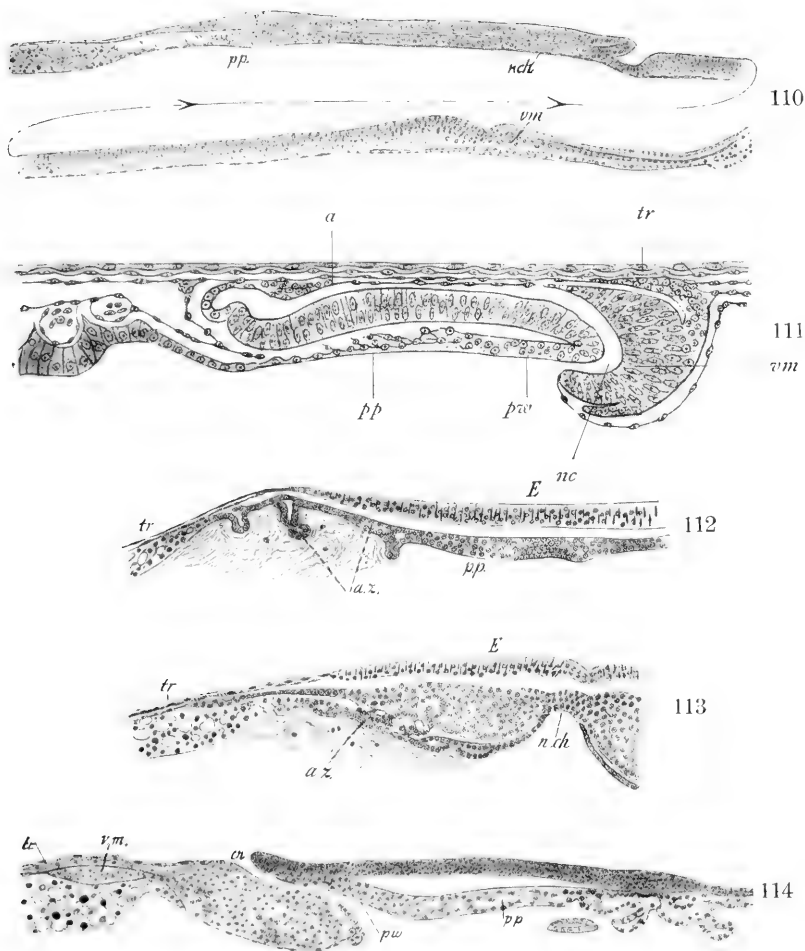


Fig. 110. Longitudinal section of a sparrow's blastodisk (after Schauinsland, 03). *pp* protochordal plate, *nch* notochord, *vm* ventral mesoblast. — Fig. 111. Longitudinal section of an early embryo of *Chamaeleo* (after Schauinsland, 03). *pp* protochordal plate, *pw* protochordal wedge, *vm* ventral mesoblast, *nc* neurenteric canal, *a* amion, *tr* two-layered trophoblast. — Fig. 112 to 114. Three sections through early blastodisks of *Sphenodon* (after Schauinsland, 03). In Fig. 114 (longitudinal section) protochordal plate (*pp*), protochordal wedge *pw*, ventral mesoblast (*vm*) and trophoblast *tr* are indicated. In this phase of notogenesis a long and distinct neurenteric canal (*nc*) is present. In Fig. 112, which is further anteriorly situated than Fig. 113, the protochordal plate *pp* is transversely cut, as well as the annular zone of proliferating entoderm *az* spreading backwards and contributing to the formation of the area vasculosa. In Fig. 113 the notochord *nch* has commenced its mediolongitudinal differentiation.

ventrally, and is found back by us in this same position, and at the same time quite marked in Fig. 111 for *Chamaeleo*.

For *Lacerta* all this is yet more distinctly brought before us in Wenckebach's well-known figures ('86) reproduced in Hertwig's Figs. 437—441.

And as to birds *Schauinsland's* longitudinal section of the sparrow (Fig. 110) leaves no doubt whatever that the phenomena are, indeed, the same here as in reptiles and mammals. Protochordal plate, protochordal wedge, lateral lips of the dorsal mouth (primitive streak), and ventral mesoblast have each their proper position, and their adequate further development.

It is especially evident in this last-named section that the so-called *Sichelrinne* has the ventral mesoblast immediately behind it, and that in this region there is another thickening of the entoderm, preceding the formation of vasifactive tissue in this region as is indicated in the diagram of Fig. 46 that was more especially designed for mammals.

In finding all this amount of correspondence between Mammals, Amphibia and Sauropsids concerning certain general features of the very earliest development of mesoblastic structures it was, of course, a great desideratum to know in how far the oviparous mammals, the *Ornithodelphia*, agreed. This paragraph was already written, and the blank caused by our ignorance on this head was keenly felt by me when the very recent memoir by Wilson and Hill on *Ornithorhynchus* ontogeny appeared in the 'Philosophical Transactions' ('07). This important paper furnishes us with the necessary data to fill up that blank, and I was only too pleased to have to re-model this paragraph as these data confirm in many respects the views here advocated, and accentuate a few points in my interpretation with quite unexpected decisiveness.

I may begin by remarking that a protochordal plate, of which mention has been made in the foregoing pages, is recognised by them as occurring in *Ornithorhynchus*, and is also designated by that name. However, the data concerning

the earliest appearance of this protochordal plate in *Ornithorhynchus* are too scanty than that I have ventured to mention it when in the preceding pages we discussed the protochordal plate. And it seems advisable on this point to await yet further researches on these rare mammals, of which it is so very difficult to obtain the required developmental stages.

As to the protochordal wedge and the ventral mesoblast of *Sauropsida* and *Ornithodelphia* some startling facts have been brought to light by Wilson and Hill, and will here be compared to what we find in reptiles, birds, and mammals higher than the monotremes.¹

The most important discovery in *Ornithorhynchus* seems to be that mesoblast formation on the upper surface of what corresponds to the mammalian embryonic shield is started at two different spots lying in the median line. In other words the protochordal wedge and the ventral mesoblast, which we have followed in all the details of their earliest origin in *Tarsius* (p. 36), and which we have there found in closest proximity (see also Figs. 48, 49, and 50) are in *Ornithorhynchus* as wide apart as is indicated by Fig. 115. In *Sauropsids*, again, they have been found confluent by successive authors, but then in *Ornithodelphia* they become confluent soon after (Fig. 116), so that there is yet room for inquiry whether perhaps certain *Sauropsids* might not in very early stages conform with *Ornithorhynchus*.

The lesson should be drawn from the arrangements in

¹ In passing, I may remark that Wilson and Hill's article is a very instructive example of the impossibility we have come to of retaining the current nomenclature, as this has gradually developed itself out of successive contributions of different authors. Only gradually can we attempt to obtain a more full comparative grasp of the subject here treated, and then such names as head-process, primitive knob and streak, and many other, prove to be a misleading encumbrance. Already Wilson and Hill do not look upon the primitive streak of mammals and reptiles as a homologous structure ('07, p. 116), whereas they propose to drop "head-process" altogether (a point already advocated by myself and others). But then they add new ones, such as archenteric plate and others, the acceptability of which will yet have to be tested and seems to me questionable.

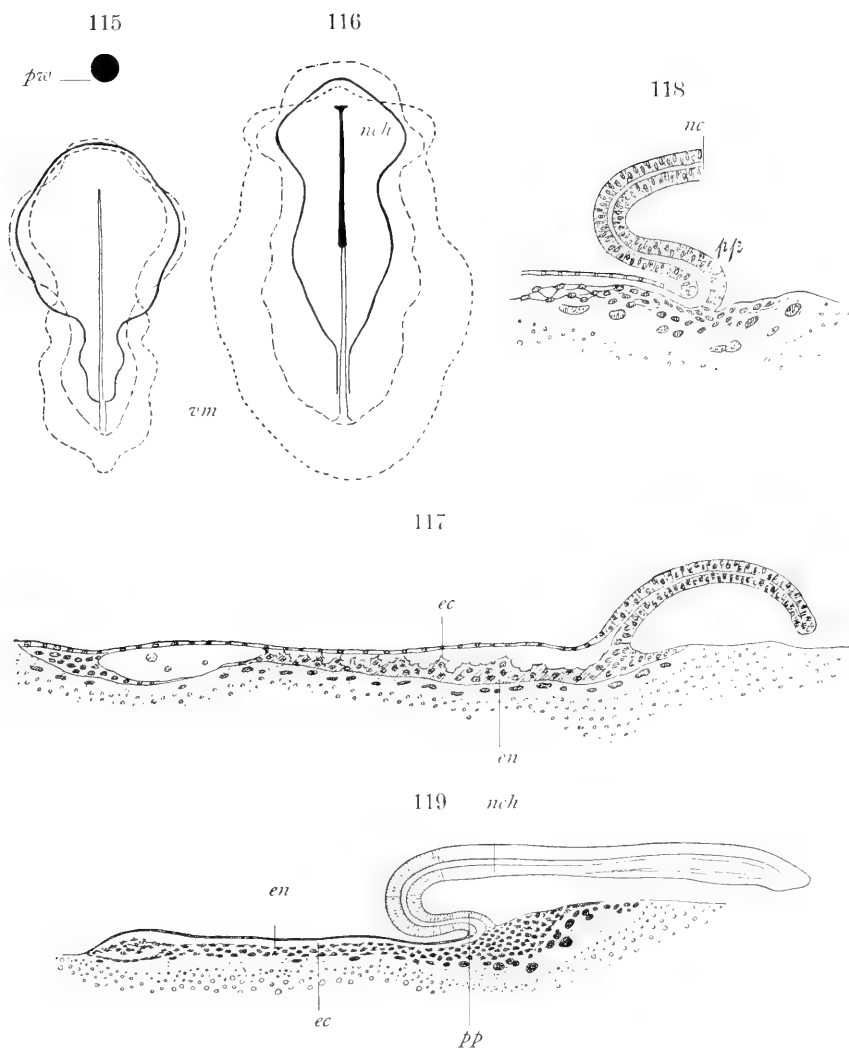


Fig. 115 and 116. Two surface views of early blastodisks of *Ornithorhynchus* (after Wilson and Hill, '07). In Fig. 115 the protochordal wedge (*pxw*) and the ventral mesoblast *vm* are yet wide apart; in Fig. 116 they are fused in the median line and proliferation takes place along the whole length of the dorsal mouth, *nch* notochord. — Fig. 117 to 119. Three longitudinal sections through consecutive stages of notogenesis of *Torpedo* (after His, '00). In Fig. 117 ectoderm (*ec*) and entoderm (*en*) have been separated by delamination and notogenesis has commenced; in Fig. 118 the folding off of the head has proceeded further; in Fig. 118 and 119 part of the protochordal plate is being lifted up from the yolk. *pp* protochordal plate, *nch* notochord.

Ornithorhynchus—for the details of which I refer the reader to Wilson and Hill's paper ('07)—that in that particular region of the embryo, where notogenesis comes about, there are multiple centres of growth. What was in the pelagic vermactinian stage of the vertebrate ancestor (Fig. 160) the dorsal mouth-slit or "Rückenmund" (from which the stomodæum [notochord] continued downwards towards the intestine while the enteric chambers preceded the cœlom) has left in the early vertebrate embryo hereditary traces of its gradual extension backwards and of its closure.¹ The proximal end of this dorsal mouth-slit is the earliest protochordal wedge, the distal end of it is our earliest growth-centre of the ventral mesoblast. Between these two there is (1) a backward growth of the protochordal wedge (above discussed for Tarsius and Amphibia); (2) a forward growth going to be confluent with the preceding and established by Wilson and Hill for Ornithorhynchus, as well as (3) lateral expansions from what may be called the lateral lips of the dorsal mouth-slit. What has been called the "Sichelrinne" is always situated at the distal end of this medio-dorsal region of proliferation, whereas what is originally the protochordal wedge (Hensen's knob) is always at the proximal extremity in the original stage. It may be said to travel for a certain distance backwards before becoming unrecognisably united to the posterior proliferation.

What has sometimes been called the archenteric cavity in the protochordal wedge (which has been compared with the archenteron of *Amphioxus* by van Beneden, who has more especially studied it in bats ['87]), what has been termed *Mesodermsäckchen* by O. Hertwig ('06, p. 828), and what has been found as a transverse slit in *Ornithorhynchus* by Wilson and Hill, and as a decided cavity by many other authors, such as Will ('90), Mitsukuri ('93), Ballowitz ('01), Wenckebach ('86),

¹ In this respect Hertwig's views can be made to fit in very well with mine, only with the difference that the "Rückenmund" is not to be confused with an "Urmund," and that "notogenesis" is not to be looked upon as "gastrulation."

Voeltzkow ('99), Schauinsland ('03) in all orders of Reptiles seems to me to be the remnant of the space which has always been included in the cœlenterate ancestors between the two lateral lips of the stomodæum of the elongating, actinia-like ancestral form. That from the wall of this cavity the notochord develops is only natural; that it communicates—be it even in a somewhat whimsical fashion and curiously intermittently—with the enteric cavity is also nothing but a hereditary reminiscence; that laterally it is inclined to tend towards communication with protosomites, as Wilson and Hill describe for *Ornithorhynchus* ('07) and Spee ('01) for *Cavia*¹ is again easily understood, if we remember that those portions of the primitive cœlenterate enteron which must have become the precursors of a cœlom (separated from an enteron) are in immediate continuity with the lower limit of the stomodæum (notochord).

The fact that this cavity, or slit, or porus offers a different extension and different shapes in different vertebrates; that in some it appears as a neurenteric canal, which in others is no more visible as an open space; that it undergoes a displacement backwards, and finally disappears, after having appeared in different parts of what will be the median plane of the back of the animal, shows that during notogenesis this important discontinuity of tissue of phylogenetic significance has also that particular variability which very old and archaic portions of vertebrate organisation so often display (e. g. epiphysis, thyroid structures, etc.).

To look upon it, as van Beneden has attempted, as the archenteron, and to degrade the original entoderm to the

¹ The very important question: in how far Wilson and Hill are right in stating ('07, p. 117) that the protosomites which they describe and figure "have nothing to do with the origination of the first definitive somites, nor are they in any way coextensive with the site of differentiation of the latter," will not here be entered upon as falling, as the superscription of this chapter indicates, outside the scope of this paper. Suffice it to say that my own preparations furnish me with most useful material for throwing light on these very obscure, but, nevertheless, all-important, points in the development of mammals, to which I intend to devote full attention on another occasion.

value of a lecithophore, is an unwarranted hypothesis in the same direction as that of the gastrulation in two phases, of which Keibel and myself were the godfathers many years ago (Keibel ['89], Hubrecht ['88]), but which we have both abandoned since.

IV. FISHES.

The early developmental phases of the germinal layers of the fishes have not been the object of personal observations of any extension on my own part, although I possess a certain number of section series both of Elasmobranchs and Teleosts.

And so I only intend, in the following pages, to give some gleanings from the literature on the subject, which appear to me to point to the possibility that the views to which investigations of the mammalia have led me may also hold good for these lower vertebrates.

Beginning with *Amphioxus*, I need only allude to Legros' latest contribution to the ontogeny of this animal, from which I have copied Fig. 120. In it we see the region marked *pp*, singled out by Legros as a part of the original entoderm of the wide-mouthed gastrula, which in *Amphioxus* is formed—in contradistinction to all other Vertebrates—by invagination, and not by delamination (cf. p. 13).

Legros ('07) and Cerfontaine ('06) are willing to adopt the essential points of Lwoff's interpretation, which has been so fruitful in setting other observers to pause and reflect. The part marked *pw* in the longitudinal section (Fig. 120) of a stage of early notogenesis should thus be looked upon as the median portion of what is Lwoff's "Dorsalplatte," and as an essentially ectodermal derivate which has come about in consequence of the growth backwards of what was originally the dorsal lip of the early blastopore (cf. for mammals with Figs. 48, 49, and 97—99 of Tarsius). During this backward growth, which does not, as is the general belief, complete gastrulation (see Hubrecht, '05), but which initiates notogenesis, the two embryonic regions become distinguishable, which, also in

Amphioxus, we may term the essentially entodermic protochordal plate (*pp*) and the (ectodermic) protochordal wedge (*pw*).

In Elasmobranchs the comparison is not either difficult or strained. We see in the Figs. 117—119 and 121—123, copied from His and Rückert, that when once the separation of the two primary layers has come about by delamination, a proliferation in the ectoderm makes its appearance (*pw* Fig. 122), which here, too, deserves the name of protochordal wedge, whereas the undoubted entodermal layer (*pp*) to the left of the figure is, and remains, the homologue of what in the mammals we have called the protochordal plate. In Fig. 123 notogenesis is in its incipient stage; in Fig. 117 it has considerably advanced, and in 118 and 119, where the headfold has made its appearance, part of what was the horizontally outspread layer (*pp*) has become lifted up from the yolk and is now that part of the entoderm which lies in front of the anterior end of the notochord, and which participates by means of its surface that faces the ectoderm in the formation of the endothelium of the heart (cf. Rückert and Mollier in 'Hertwig's Handbuch,' Bd. I, 1; II, p. 10; 33, 34).

Perfectly similar occurrences were formerly described by me ('02, Pl. IX, figs. 72-74) for 'Tarsius, and Figs. 98, 99 should be compared with those here given for the Elasmobranchs.

As to the presence in Elasmobranchs of a ring-shaped extent of entoderm which contributes to the formation of the mesenchyme, out of which the vascular system is going to take its origin, we find the most reliable data in Rückert and Mollier's extensive treatise above cited. Their figure 777, here reproduced as Fig. 124, is especially instructive, and it will suffice to refer to that important article in 'Hertwig's Handbook.' It will there be seen that also by the presence of a circular region of mesenchyme-producing entoderm the early Elasmobranch stages resemble the mammalian. Although in Rückert's article a certain reluctance is unmistakable to admit the value of his conclusion as to the entoblastic origin

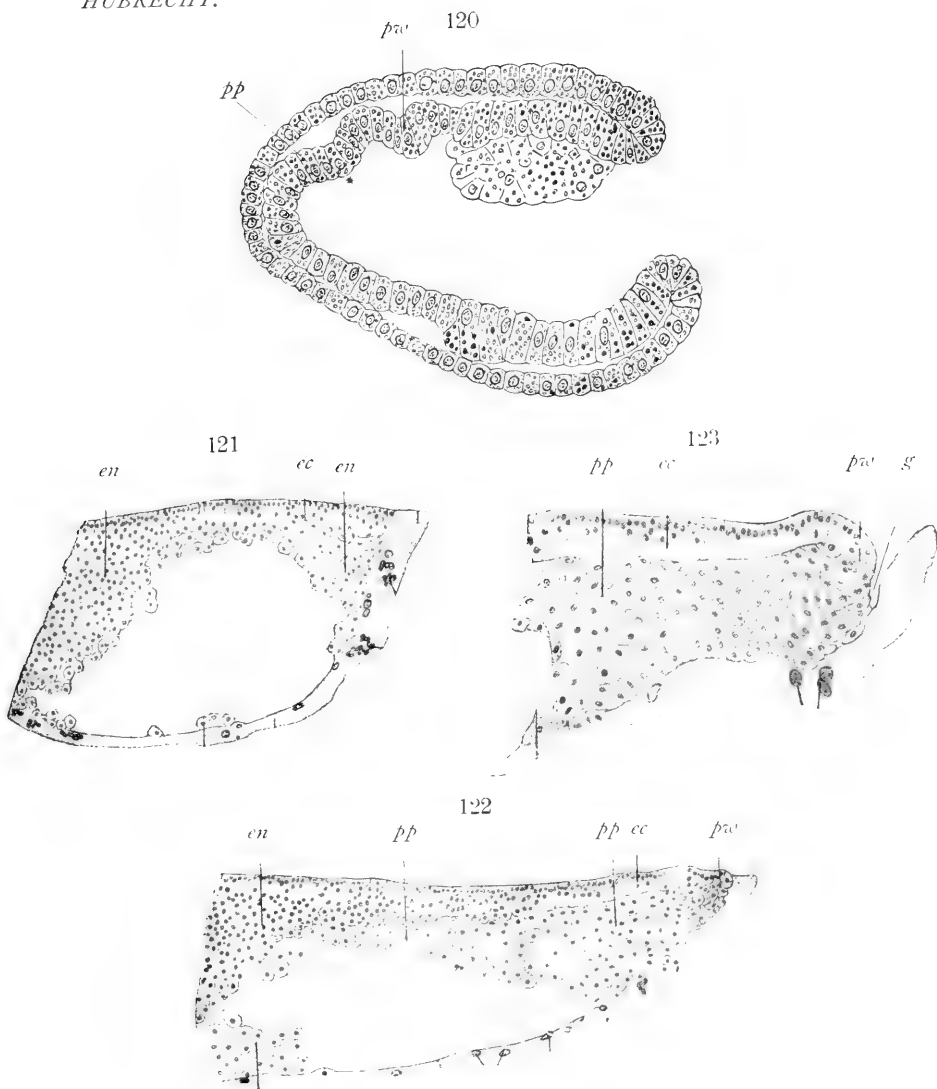


Fig. 120. Longitudinal section of *Amphioxus* (after Legros, '07). Noto-genesis has advanced considerably. At * Legros accepts a separation between the lower entodermal layer, originated by invagination and the layer to the right of * which he accepts on Lwoff's example as a product of ectodermal proliferation. * would then separate: to the left: protochordal plate *pp*; to the right: the protochordal wedge *pw*, spun out. — Fig. 121, 122 and 123. Three longitudinal sections through blastodisks of *Pristiurus* (after Rückert, '06). In the two first gastrulation by delamination has come about: *cc* ectoderm, *en* entoderm; in Fig. 122 the protochordal wedge *pw* has fused with the entoderm *pp*; in Fig. 123 notogenesis has definitely commenced. *g* incipient gut cavity.



of the blood-producing mesenchyme, still *de facto* he is quite outspoken about it. His conclusion (l. c., p. 1095) that it is not of any importance, whether the blood anlagen of the Selachians are meso- or entoblastic is not ours, because we have in the first part of this chapter demonstrated that a precise analysis of the mesoblast formation also in mammals may considerably contribute to render comparison of the various classes more easy. That in Elasmobranchs, as in mammals, a very early fusion comes about between Rückert's peripheral and axial mesoblast, and that, after this has come about, an end is made to further unravelling is certainly true, but it may not withhold us from laying all the more stress on the necessity of comparing the very earliest stages with the utmost closeness.

The focus of proliferation corresponding to what we have called the ventral mesoblast in the Mammalia and the Amphibia must be sought for in the Elasmobranchs in the tail swellings. I do not intend to enter into further comparison here, but will postpone this to a later paper where the stages after the development of the mesoblastic somites will be discussed.

Among the Teleostomes we find developmental stages which have again a different character from what we noticed in Elasmobranch fishes. In many cases the eggs are not meroblastic, and then a more or less close comparison is possible between their development, and that of the Amphibia above discussed. So it is with the Sturgeon's eggs and with those of the Dipnoi: *Ceratodus*, *Lepidosiren*, and *Protopterus* (Fig. 125). Other comparisons with the development of the lamprey suggest themselves, and have already been pointed out by different authors. As to the external features of this development, the Rückenrinne, which Semon has figured for *Ceratodus* (Fig. 126), and which has also been noticed in Urodeles by Braus seems to me to be simply explained if we look upon it, not as any remnant of a blastopore (Urmund), as Semon ('93, pp. 37—39) proposes, but as the last reminisc-

ence of what was the ancestral "Rückenmund" of a more actinian-like ancestor.

In a later paper Semon ('01, p. 317) retracts his original comparison, arguing that the gastrulation process has already ceased before the "Naht" becomes visible. Here again the false interpretation of the gastrulation process (see Hubrecht, '05) has misled Semon, whose original interpretation can be adhered to if we substitute notogenesis for gastrulation, and dorsal mouth (Rückenmund) for blastopore (Urmund).

Protochordal plate and protochordal wedge in their mutual relations may be considered identical with what we discussed for the Amphibia.

The ventral mesoblast, which, in *Petromyzon*, does not appear as so distinct a median and early proliferation of the ventral lip of the blastopore such as we meet with it in many amphibians, has not either that character at so early an age in Dipnoi and Ganoids; the homologue of it is found in the Schwanzknopf, as we also saw in Elasmobranchs. And Fig. 87, for *Amia*, indicates, moreover, if we compare it with Fig. 99 for *Tarsius*, that the intestinal continuation *al* in both figures is of the same order, although in the mammal it is no slit, but has become a tube. Moreover, both of them may be brought into line with Kupffer's vesicle in Teleosts.

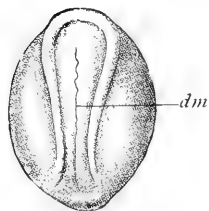
In many respects the development of the Teleosts offers peculiarities not met with in the Vertebrates hitherto discussed. The process of notogenesis has certain remarkable points of comparison with what we notice in many Amphibia. Ziegler has, in his 'Lehrbuch der vergl. Anatomie der niederen Wirbeltiere' given four coloured diagrams (Figs. 11—14) to accentuate the extent of this comparability, and says in his text (p. 182): "The ventral border of the blastoderm advances quite in the same way as does the ventral point of transition between the smaller and the larger cells in *Rana* and *Triton*."

However, there are numerous points of divergence and the different authors who have of late occupied themselves with

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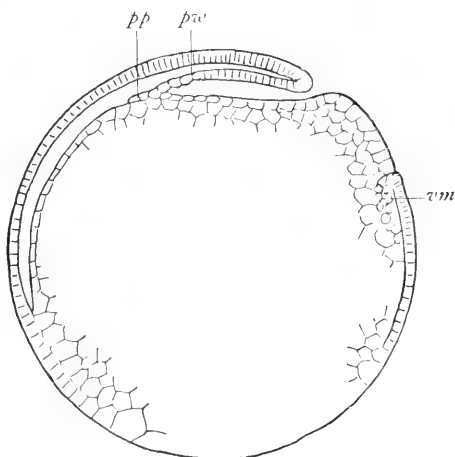
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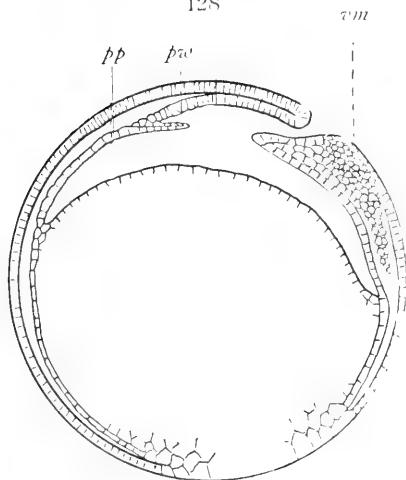


Fig. 124. Transverse section of the right half of an early embryonic shield of *Torpedo* (after Rückert and Molier, '06). The participation of a region of entoderm *az* towards the formation of blood and blood-vessels is here indicated. — Fig. 125. Longitudinal section of *Lepidosiren* (after Graham Kerr, '01). cf. *Amphibia* (Fig. 79). *pp* protochordal plate, *pw* protochordal wedge. — Fig. 126. The dorsal raphe (dorsal mouth, „Rückenrinne“) *dm* of *Ceratodus* seen from above (after Semon). Fig. 127 and 128. Two diagrammatic longitudinal sections of stages in the notogenesis of *Amphibia* (after Ziegler, '02). *pp* protochordal plate, *pw* protochordal wedge, *vm* ventral mesoblast.

Teleostean ontogeny are far from being in entire accordance on many points. Swaen and Brachet ('99, '04), H. Wilson ('91), Sumner ('04), and Boeke ('02, '07) are among the authors who have lately considerably furthered our knowledge of Teleostean development. The latter author more in particular, who adopts my views concerning gastrulation and separates that process from what I have proposed to designate as notogenesis, gives certain figures which point in a direction that may finally lead to a more close comparison of the processes in Amphibia and Mammals on one hand, in Teleosts on the other. I copy a few figures from his latest papers in my Figs. 88 and 89 to elucidate how I imagine that it may perhaps later be possible to distinguish also in Teleosts a protochordal plate (*pp*) and a protochordal wedge (*pw*) standing in the same relation to each other as in mammals. The homologue of the protochordal plate can no doubt be detected in a portion of the periblast, to which layer, since Boeke's detailed investigations, participation in the formation of embryonic cells can no longer be denied. It is certainly striking that this participation is of a nature that would bring it in one line with those processes which we have above noticed, both in the protochordal plate and in the annular zone of entoderm, that is so closely allied with vasifastive phenomena. On the other hand the protochordal wedge, as a downward proliferation of the ectoderm increasing in length by a backward growth of the blastoderm and obtaining after some time a new coating of entoderm-cells below it, is quite exceptionally evident in Teleosts (Figs. 88, 89).

The focus of formation of the ventral mesoblast is in Teleosts originally far apart from the protochordal wedge, but fuses with it when the yolk has been entirely overgrown, and when what was at the outset the anterior ring of the blastodisc has coalesced from behind with the prostomial thickening. This prostomial thickening of Teleosts has since Boeke's researches ('02*b*) to be looked upon as an entodermal (periblastic) proliferation, and is by me homologised with that proliferation in the entoderm of mammals which occurs

in the posterior region of the annular zone described in Chapter II, paragraph 2*b*, and diagrammatically represented behind the protochordal wedge in Fig. 46. The fact that in and behind this entodermic proliferation Kupffer's vesicle is developed seems to further confirm that homology, as will be noted below. This vesicle becomes apparent when the yolk has become quite enclosed by embryonic tissue.

I have pleasure in noting that my identification of Kupffer's vesicle in Teleosts with the allantois in primitive mammals (a comparison which Kupffer himself did not fail to make) is accepted by Boeke. A comparison between Figs. 90, 128, and 63 will further elucidate the chain of thoughts here implied. And I may call particular attention to Swaen and Brachet's article ('04) and their figures 58 to 77 (Pl. XV) of *Trutta fario* in order to show how these authors have established a close connection between this cylindrical posterior continuation of the entoderm, which goes by the name of Kupffer's vesicle, and the production of vascular tissue (Swaen and Brachet's *Lame vasculaire*, *L.v.*). I invite close comparison with what I have written about *Tarsius* on p. 45, and with Fig. 102 which was given of that mammal, and have no doubt that if we compare Kupffer's vesicle not with a free allantois of reptiles and most mammals, but with the incipient allantois met with in Primates, many apparent objections to such comparison will fall to the ground.

SUMMARY OF CHAPTERS I AND II.

Before the ectoderm and the entoderm have become differentiated from each other there is in mammals a distinct larval cell-layer surrounding (as soon as the cleavage of the egg has attained the morula stage) the mother-cells of the embryonic tissues. This layer, to which the name of trophoblast has been given, and which is, phylogenetically, an ectodermal derivate, contributes towards the formation of chorion and amnion, and is shed at birth. The mother-cells of ecto- and entoderm enclosed within the trophoblast, and at one point

in immediate contiguity with it, separate into ecto- and entoderm by delamination.¹

The result of this delamination is the mammalian gastrula—sometimes characterised by the temporary presence of an actual blastopore—which very soon undergoes a series of developmental changes (different from gastrulation) to which, in this incipient stage, the name of notogenesis may be applied. The tissues further forwards, that were the first to appear, are simultaneously contributing to what may be called kephalogenesis. The budding of the trunk from what becomes the extreme forward region of the head is designated by these two terms.

Before notogenesis has commenced, a posterior portion of the ectodermal embryonic shield (immediately behind the spot where the blastopore is actually or only virtually situated) is told off as the mother-tissue of what will be the ventral mesoblast. When notogenesis is inaugurated it does so by a marked median and ventral proliferation of the ectoderm in front of the posterior region just alluded to. This proliferating downgrowth (the protochordal wedge) becomes confluent with the entoderm and fuses with a proliferation in it (the protochordal plate).

Both proliferations are centres of origin of mesoblastic and mesenchymatic tissues. The antero-median entodermal proliferation, called the protochordal plate, is in continuity with a ring-shaped area stretching sideways and closing behind, below the ventral mesoblast. This ring is the place of origin of blood and blood-vessels. In certain mammals it contributes in its hinder portion to a very early vascularisation of the trophoblast, thereby calling forth a connective stalk (*Bauchstiel*), in which a remnant of entoderm, drawn out in tube form, is the first indication of what in less primitive arrangements has come to be the allantois.

¹ The two germ-layers (ectoderm and entoderm) of all the Vertebrates arise by delamination; only in *Amphioxus* they owe their differentiation to a process of invagination so common among invertebrates.

CHAPTER III. DIPLOTROPHOBLAST=SEROUS (SUBZONAL) MEMBRANE,
CHORION, AMNION, ALLANTOIS AND UMBILICAL VESICLE IN
ONTOGENY AND IN PHYLOGENY.

In the second chapter of this treatise we have discussed the facts that have convinced us, that the very early phases in the development of mammals are characterised by the presence of what we look upon as a very early larval envelope: the trophoblast. We have on p. 15 tried to imagine how this larval envelope might have evolved out of arrangements that are met with among invertebrates.

We have also hinted at a possible simplification of the current views concerning the phylogeny of the foetal envelopes of the higher Vertebrates, views which, at present, are neither satisfactory nor unanimously accepted.

But we have not yet given any detailed account as to how we have to picture to ourselves the phylogenetic processes by which out of this primitive trophoblast the different foetal envelopes have evolved, and how it has come about that some of those have been vascularised in diverse ways and have thus laid the foundation for the phenomena of placentation, so intimately related to the higher development which characterises the mammalia as against the lower Vertebrates.

I must begin by stating that much of what is going to be brought forward in this chapter is an attempt at bringing together in the light of an hypothetical interpretation facts that have up to now been insufficiently understood or overlooked. A solution that is anything else than hypothetical can, of course, never be reached.

1. Chorion and Amnion.

The amnion is a membranous envelope which we encounter in all Mammalia and Sauropsida and of which no traces are found amongst Amphibia and Fishes, the two latter being distinguished as Anamnia from the former, the Amniota.

Various views have been expressed concerning the phylo-

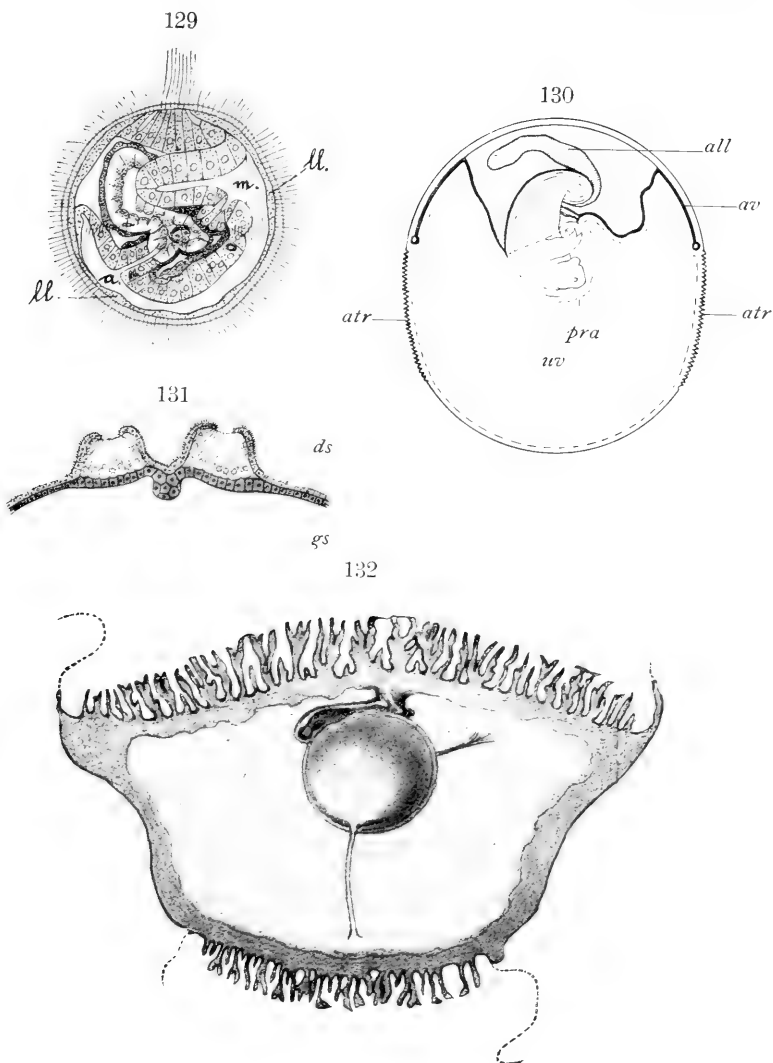


Fig. 129. Longitudinal section of a larva of *Sipunculus nudus* with its external larval layer *ll*, which, later on, is shed; *m* mouth, *a* anus (after Hatschek, '84). — Fig. 130. Longitudinal diagrammatic section of an embryo of *Dasyurus* (after Hill, '00). *all* allantois, *av* area vasculosa on umbilical sac *uv*, *atr* region in which the trophoblast shows phagocytic properties. *pra* proamion. — Fig. 131. Section through part of the „Deckschicht“ (*ds*) with local proliferation of same in the frog. *gs* „Grundsicht“ (after Assheton, '96). — Fig. 132. Longitudinal diagrammatic section of the blastocyst of a Catarrhine monkey (*Cercocebus cynomolgus*) with dorsal and ventral placenta (after Selenka, '91).

genesis of the amnion. And it must be recognised that the appearance—all of a sudden—of this useful and complicated foetal investment—though it is only present during the few weeks or months between early ontogeny and birth—is strictly comparable, both as regards its constituent layers and the way it comes into existence wherever we find it. This natural phenomenon must have its natural evolutionary explanation for whosoever wishes to be guided by evolutionary principles in his interpretation of nature. The explanations given, and which we owe to Haeckel, van Beneden and others are, however, as we shall see, untenable. I have held this view long ago ('95), and have made another attempt at solving this evolutionary riddle, but find that a repetition of my argumentation towards an alternative solution of this intricate problem may not seem out of place.

Now it is a very queer point that two of the foetal envelopes, the amnion and the serous membrane, seem to be so intricately interlocked with each other as far as their first appearance goes, that the serous membrane, as Schauinsland—the author of the chapter on foetal membranes in Sauropsida in Hertwig's new handbook—puts it, owes its origin to the outer pleat of the amnion fold, but later increases in size by a process which splits it off from the umbilical vesicle. Of late the name amniogenetic chorion (Bonnet) has been introduced for mammals in the place of serosa, thus underlining the supposition that it owes its origin to the amnion.

We would expect a foetal envelope of the importance of the chorion in Primates and of the subzonal membrane or serosa (diplotrophoblast) in other Mammalia and Sauropsida to have a phylogeny of its own and not to be a by-product of a folding process that is typical for the Sauropsida, but is absent in representatives of many orders of Mammalia.

A hypothesis which would separate the phylogeny of chorion (serosa) and of amnion would thus in itself appear to be more acceptable and might prove to be a better guide to the understanding of those mammals that have no amnion-folds (Cavia and other rodents, Pteropus, Galeopithecus,

Erinaceus, Gymnura, monkeys and man) than one which is obliged to derive the amniogenesis in the latter mammals from the phenomena which we notice in Sauropsida and a number of mammals by an obscure process of cenogenesis.

Such an alternate hypothesis I have advocated ('95B) more than thirteen years ago, and will here state it anew with certain additional facts in its support.

The starting-point for this hypothesis, which will enable us to admit a separate phylogeny for chorion and for amnion, was the fact that we have observed in all mammals the presence of an actual foetal envelope—the trophoblast—long before the appearance of anything like the amnion. And secondly the other fact that this early foetal envelope is bodily transformed into what is called the chorion, diplotrophoblast, serous membrane or subzonal membrane in later stages of development. There can thus be little doubt that we now have to turn the tables and must no longer look upon this outer envelope as a by-product of the amnion, but must, on the contrary, ask how has the amnion come to be developed out of or by the side of the older foetal membrane or embryonic envelope, the trophoblast?

Leaving aside the interesting, but at present quite unsolvable question whether vertebrates have ever existed in older geological times that possessed a trophoblast but were not yet provided with an amnion (a question which is justified as soon as we have established that the link between amnion and serosa is not as indissoluble as the modern embryological text-books will have it), we will now proceed to investigate such cases in which the independent development of chorion on one side, amnion on the other is yet particularly evident.

Of such cases I would cite some of those that have been already named above and that occur in different orders of mammals. We begin with an extreme case as is that of *Cavia*. The trophoblast and that proliferating portion of it which is known as the Träger are quite independent of the embryonic knob at a very early stage (see Figs. 24 and 25). In the embryonic knob, after the mother-cells of the

endoderm have become separated from it, a cavity arises, the lower surface of which becomes the entodermic shield, the upper surface the inner lining of the amnion, which is thus a closed cavity from the very beginning.¹

Cases of a less extreme nature are met with in other Rodents, and have been described in detail by Selenka ('83, '84) and others. Thus for mouse and rat, for *Arvicola* and others, the cavity which in later stages is called the amnion is never in free communication with the space outside the trophoblast, but is always intra-trophoblastic. There is thus no necessity for a gradual meeting of folds in order to delimitate the amnion cavity which is an enclosed space from the very earliest (Figs. 27, 28). What is known and figured as the head-fold and tail-fold of the amnion in the mouse (Fig. 28) must be more fully investigated before comparing it with the same structures in Sauropsida. Moreover, these folds in the mouse, when once confluent, do not form a boundary between the amnion cavity and the outer world, but between two cavities inside the trophoblast, one of which is the amnion. Among the Rodents the case of *Lepus* is particularly instructive. I have already called attention to it in an earlier publication ('95 B), and have there demonstrated that the so-called Rauber cells form part of the trophoblast, with which they are continuous (Fig. 23). In *Lepus* the trophoblast does not open out to bring the embryonic ectoderm to the surface, but the trophoblastic cells above the latter flatten out and disappear in the way of the "Deckschicht" of the Amphibia. This is not a primitive but probably a secondary arrangement, as may also be inferred from what Lee has found in another rodent (*Ammospermophilus*), which may be

¹ In former publications ('95 B, p. 25) I have more than once suggested that the amnion formation in man and monkeys (not known by actual observation) was probably of the same order as that of *Cavia*. While correcting this proof I became acquainted with the interesting early human ovum which was demonstrated at the Berlin Congress of Zoologists in April, 1908, by Drs. Bryce and Teacher, and I feel confident that it goes a long way towards confirmation of this suggestion, if later finds will prove it to have been normal.

said (Fig. 15) to be intermediate between the entypic, such as it is represented in many rodents, ungulates, and insectivores (Figs. 13, 17, 29—32), and the flat embryonic ectoderm of *Lepus*, *Sorex*, and others.¹

A case which as far as the amnion is concerned offers great similarity to that of the Guinea-pig is that of the flying dog (*Pteropus*), where Selenka and Göhre ('92) noticed a closed amnion from the very first. It develops into the definite amnion by a simple process of extension and moulding, and exists as a closed sac of ectodermal constitution long before a mesoblastic layer comes to duplicate its wall (Figs. 22, 71—73).

A very similar arrangement is met with in the yet much more primitive *Galeopithecus* (Figs. 41, 42), which, however, I have not yet found occasion to describe in detail.

Another very instructive case is that of *Erinaceus* and *Gymnura*. In one of these two genera we have described how the early blastocyst is characterised by the possession of an embryonic knob from which the entoderm is so quickly separated off that the details of its earliest development have not yet come to light quite sufficiently. But at the same time the remaining embryonic ectoderm takes somewhat more time to become separated off from the trophoblast than in other mammals. And when it does it is by the appearance of a cavity between what is going to be the ectodermal shield and what will remain the trophoblast that the amnion-cavity is heralded into existence (Figs. 33—37). Here again it is a cavity that appears as such that strikes us as the dominant feature in the phenomenon. I have elsewhere demonstrated ('95 B) that if we have to choose what is the more probable earliest appearance of the amnion, as a closed cavity or as set of folds by the meeting of which a cavity is going to be enclosed above the dorsal surface of the embryo, there is undoubtedly—speaking as an evolutionist—a heavy

¹ The exact details of the formation of the amnion in *Ammospermophilus* with respect to the exact derivation of its inner (epiblastic) layer should be looked forward to with interest.

balance in favour of choosing the first eventuality. Principally because only in case these have been the actual phylogenetic steps can we conceive that the amnion on its very first appearance was of immediate significance to the embryo, as a sort of water-cushion, shielding the embryonic rudiment—already at its very earliest appearance—from external pressure or mechanical insult. We have now seen that not only does the amnion appear as a closed sac from the very earliest in very numerous cases in different orders of mammals (to the list already given the monkeys and man should yet be added), but that in this case an explanation of its earliest origin is not far to seek. We have indeed admitted that the trophoblast is an early larval envelope by the presence of which a chorion or serous membrane is predestined to make its appearance sooner or later. From this larval envelope—also in the case of *Nemertea* and *Gephyrea* (Fig. 129)—the embryonic ectoderm has to become segregated in one way or another, as also the amnion is being originated in different ways. We know from the examples we possess amongst *Nemertea* of the *Pilidium* larva and the Desor's larva that at one time this segregation takes the form of a splitting process (Desor's larva) when (as is the case in the dorsal plate of that larva which I have formerly ['85, Figs. 53a, 95] described) the plate of future embryonic ectoderm provisionally remains attached to the larval envelope along a circular line of attachment much in the same way as we see the embryonic ectoderm of the hedgehog attached to the trophoblast (Fig. 37) with the closed amnion-cavity above it; whereas at another time (the lateral plates of the Desor larva or the embryonic plates of the *Pilidium*) the separation of the definite ectoderm from the larval layer takes place by a process of invagination, during which that portion that is destined to become the outer wall of the embryo sinks away from the level of the larval surface into the cavity enclosed by that surface and develops further in this more sheltered position. In this latter case a circular fold ensures the continuity between larval and definite ectoderm, and only when the

folds meet has the surface of the *Pilidium* become separated from the future body-wall of the embryo and are these two separated by a closed cavity which, also in the case of the *Pilidium* larva, has for many long years borne the name of amnion cavity. It makes no difference that in the *Pilidium* the process occurs at four different spots, the products of which fuse later. A. Willey ('98) has speculated upon similar relations between arthropod embryos and their larval envelope, also designated as amnion in *Peripatus*, *Lepisma*, *Gryllus*, *Forficula*, and others; has rightly interpreted the direct comparability with the vertebrate trophoblast, and has looked upon it (as I have done ['95 B] for vertebrates) as an adaptation to a viviparous habit acquired by the terrestrial descendant of an aquatic ancestor.

And so not only can we link on the larval trophoblast to cases met with amongst the invertebrates, but even for the development either of a closed amnion or of an amnion arising by circular folds (perhaps in the case of the invertebrates also a secondary modification) do we find examples in the invertebrate kingdom.

It seems to me that in the case of vertebrates we may be content to say: (*a*) that wherever an amnion is met with it is the sequence of the separation of the embryonic ectoderm from the larval envelope; (*b*) that this larval envelope (trophoblast, giving rise to chorion, diplotrophoblast, or serosa) is always antecedent to and must be older than the amnion; (*c*) that the actual separation of the embryonic ectoderm from the trophoblast can be witnessed in those mammals where the amnion is from the first a closed cavity; and finally (*d*) that in those cases, both among mammals and Sauropsids where we do not notice a direct separation between embryonic ectoderm and trophoblast in consequence of which an amniotic space arises, we see the amniotic cavity appear at a later stage, thanks to a folding process, which may be entirely restricted to the ectodermal tissues, and for the formation of which the presence of mesoblast is in no way required.

Returning to the hedgehog for finding a reasonable explanation of how the folds may have arisen, when the amnion was no longer formed as a closed cavity *ab initio*, we see that here and in the bat a phenomenon occurs that does throw light on this point. We see (Fig. 38) that when a certain stage of development has been reached the circular rim of attachment of the ectodermal shield to the trophoblast shows a tendency to travel upwards. I have formerly ('95 B, p. 25) ascribed this to a splitting in the deepest trophoblastic layers. I now feel inclined—as I did in a yet earlier paper ('89)—to see the first step in this direction in a more direct co-operation of that rim of the embryonic ectoderm itself, which travels upwards along the surface offered to it by the massive blood-laden trophoblast (Hubrecht, '89 p. 374, Pl. 25, fig. 51). The annular zone of attachment thus becomes more and more restricted, and when finally it disappears a separation between amnion and trophoblast is at that same moment brought about. Whether mesoblast has occasion to extend itself in the region between these two is a question in no way of vital importance for the amniogenesis, as is also demonstrated by the peculiar way in which the amnion arises in *Chamæleon*, a sauropsid which, in this respect, undoubtedly reveals primitive characters. As such we reckon the fact that the amnion has, on starting, no lining of mesoblast, and that it has the shape of a ring-fold (Figs. 75, 76) closing about the middle by a uniform constriction not yet differentiated into head-fold, tail-fold, and side-folds.

This amnion fold of *Chamæleo* has an outer plait of trophoblast, an inner plait of embryonic ectoderm, the two growing independently and passing into each other at the rim of the fold where—as in *Sphenodon* (see also Figs. 77 and 78)—lay in a somewhat earlier stage the potential line of demarcation between embryonic ectoderm and trophoblast alluded to above. These cases of reptiles are thus connected with that of the hedgehog above noticed and with that of the bats so very clearly figured by Duval ('99, Figs. 96, 102, 117,

123, 132). These latter figures, compared to Figs. 50, 57, 68, 73—76, 82, 85 on Duval's Pls. 2 and 3, make it clear to us how a case of closed amnion formation, as it is offered by *Galeopithecus* and *Pteropus* (and as it is virtually present in the very early bat stages (Duval's Figs. 50 and 57), can gradually become converted (in the other bats) in one in which the closed amnion only comes into definite existence through the gradual uprising of a rim, the outer wall of which is trophoblastic, the inner one a derivate of the embryonic ectoderm.¹

Many figures (8*a*, 13—17, 20, 23, 30—32, 37) have shown that the early separation between trophoblast and embryonic knob takes place in the most various ways. And that even in one and the same species, as, for example, *Tarsius*, the separation may come about earlier or later (cf. Hubrecht, '02, Pl. II, fig. 38, *a—e*; with Pl. VI, figs. 49, *a*, *b*, and 50 *a—c*). This later appearance calls forth a stronger resemblance between *Tarsius* and such cases as *Pteropus* or *Cavia*. At all events, it is this very early process of separation of what will be the embryonic tissues from the trophoblast that goes parallel to ontogenetic processes in the invertebrates to which we have called attention (*Pilidium*, Desor larva) as showing us the earliest causes of amniogenesis. Such cases as *Tupaja* (Fig. 30) and *Cervus* (Keibel, '99), and lately again *Sus* (Fig. 17) are particularly instructive. What Selenka has designated by the name of *Entypie* is—from our point of view—no secondary phenomenon, but one which repeats very primitive features of separation between embryonic ectoderm and larval envelope in invertebrate ancestors.

The formation of a proamnion is a phenomenon which has no significance at all for our considerations concerning the phylogeny of the amnion in general. It is a temporary

¹ I call particular attention to Duval's ('99) Figs. 96 and 102, and feel too late, while correcting the proof, that I ought to have copied them in this paper, particularly because the independent growth of the trophoblastic (outer) and of the ectodermal (inner) plait of the amnion-fold is there so extraordinarily clear.

structure met with in many mammals and sauropsids where a circular region of the blastocyst in front of the head remains without mesoblast. Into this the front end of the embryonic body curves down and is temporarily sheltered. This envelope thus consists of ectoderm and entoderm only (Fig. 130). It is during further growth of the embryo gradually reduced; the head is withdrawn from it; mesoderm gradually appears between its layers, and when the embryo is ready it has entirely lost its proamniotic covering layer which is finally flattened out.¹ Thus the explanation of the amnion which appears furthest from the truth is that of Selenka ('91, p. 186), who has expressed the opinion that the amnion arose out of a double source, and that the two Anlagen of both amnion and proamnion were finally fused into one. The true interpretation must decidedly start from quite different considerations as were developed above.

¹ The explanation of the first phylogenetic origin of a proamnion has not yet been attempted; generally it has been ascribed to rapidity of growth, which caused the embryo's head to become temporarily imbedded in its own umbilical vesicle. But then the Primates, who have undoubtedly the biggest heads—comparatively!—have no proamnion.

My own explanation is a very different one, and starts, not from yolk-laden eggs of Sauropsids, but from early viviparous protetrapods which must have preceded (see p. 15) both Sauropsids and Mammals. Some of these have obtained direct vascularisation of the trophoblast by umbilical vessels; a great many others have departed from this very direct line of perfect vascularisation, have given up the early "Haftstiel" (the homologue of which reappears in the allantoidean attachment), and have at an early stage utilised their area vasculosa on the umbilical vesicle for establishing intercourse with the mother. Thence arose a temporary omphaloidean placentation. After a time, however, the disadvantages of this system of vascularisation during the further increase in size of the embryo became evident. Not, however, before arrangements had come into existence by which the omphaloidean placentation could remain in function as long as possible. Of these arrangements the most important is no doubt the growth of the head down into the umbilical vesicle, with, as result, the formation of a proamnion. It reaches its maximum in the Didelphia which, after having given up allantoidean placentation (yet persistent in *Perameles*), enjoy omphaloidean attachment for a short time, and then come into the world under the quite specialised conditions that are so characteristic for the Marsupials.

We can now understand how the particular mode of amnion development as we find it in birds, and more especially in the chick (which was naturally the type upon which all speculations concerning the amnion were originally based, no other being sufficiently known) has given rise to that erroneous conception of the amnion as the first cause of the production of the serous membrane. The error was all the more explicable, but at the same time all the more tenacious because in the chick the existence of the trophoblast as an extra larval envelope is not in any way evident (see p. 20). It is only by placing together all the transition stages from the more primitive mammals to the Ornithodelphia and the Sauropsida, that we can succeed in demonstrating how it comes about that the ectodermal layer of cells which in the latter gradually travels round the yolk and finally encloses it is not primarily the radial extension of the ectoderm, *sensu strictiori*, of the shield, but that it owes its origin to what we have learnt to distinguish as an enveloping layer, which has lost its significance in the oviparous sauropsids, and can only be seen in its full detail in mammals.

A yet further reduction, or, to put it more correctly, a reduction in yet another direction than what we notice in Sauropsida is presented by the trophoblast of the Amphibia. Not in all, but in very many of these the development is characterised by the fact that at a very early period the outer ectodermic layer of the young embryo is so visibly different from the subjacent ectodermal cell-layers that it is distinguished by the name of "Deckschicht" from the latter which are termed the "Grundschicht." Moreover, the cells of the Deckschicht proclaim their transitory and larval significance yet further by the fact that they disappear in later developmental stages, and that it is only into the constitution of peculiar larval organs that they play any part. So in that of the sucking disc, and in that of what are considered as larval olfactory organs (Fig. 131).

This then is a decidedly transitory, we may even say larval layer. On a former occasion ('95) I have already committed

myself to a comparison between it and the mammalian trophoblast. I have since, in a recent publication ('07, p. 60), more distinctly accentuated that I would never look upon the Deckschicht of the Amphibia as having been the first starting-point of what afterwards becomes amnion and chorion of the higher mammals. We may safely say that Deckschicht and trophoblast are homologous and of similar descent, but we cannot at present fully picture to ourselves what has been the arrangement of the larval envelope in the common parent form from which both have derived. There is no doubt that the viviparity in those vertebrates that have become the higher mammals has contributed towards making the trophoblast ever so much more conspicuous. But whether for the Amphibia we may also assume that in past times the trophoblast was more conspicuous in very early stages and enclosed an embryonic knob, such as we notice in mammals, cannot be decided for the present. Suffice it to say that some few observations would seem to point in this direction. I do not, however, wish to develop these at present, considering the very hypothetical nature of the ground we are here treading on.

It should, however, be immediately observed that if we are willing to admit the homology of the amphibian Deckschicht with the mammalian trophoblast, we must then unhesitatingly go one step further.

For there is no reason why we should not consider in that same light the Deckschicht which we encounter in *Ceratodus* (Semon, '93, '01), in *Lepidosteus* (Dean, '95), in *Acipenser* (Salensky, '80, '81), representatives respectively of Dipnoi and of Ganoids; nay, we are thus insensibly led to consider the Deckschicht of the bony fishes (which is so well known a particularity of this class of Teleostomes) as also included in the group of phenomena about which we are here attempting to generalise.

And it then, of course, strikes us, supposing all these different outer covering layers during early larval life to be the remnants of an early larval envelope, of which we find no

trace in *Amphioxus*, the *Cyclostomes*, and the *Elasmobranchs*, that the deep significance hitherto attached to the foetal membranes as a means of subdividing the vertebrates into the primary groups of *Amniota* and *Anamnia* runs great risks of losing much of its significance.

We have seen that the name *Amniota*, as against *Anamnia*, was not well chosen if we consider, as I have advocated, that not the amnion, but the chorion, is the primary foetal membrane, and that the name of *Choriata*, as against *Achoria* would even have been better; that of *Allantoidea* as against *Anallantoidea* being yet more defective, as we will see later on.

But now, considering that the chorion is only a derivate of the early larval envelope that we have called the trophoblast, and that of a trophoblast traces are met with among *Amphibia*, *Dipnoi*, *Ganoids*, and *Teleostomes* in general, the more important division should not come to lie, as is at present the case, between the *Sauropsida* on one side and the *Amphibia* on the other, but between those vertebrates in which a larval envelope, or traces of it, are found and those in which such traces are absent.

We have seen, by the example of birds and reptiles, that it is not always easy to detect traces of the trophoblast, which for various reasons is not always quite as distinct as it is in mammals. And so even when among vertebrates we have descended downwards as far as the *Elasmobranchs* and the *Cyclostomes*, the possibility of last traces of trophoblast and *Deckschicht* having been obliterated cannot be denied. Still, on quite different arguments supplied by comparative anatomy, we must recognise that the line here drawn seems to correspond with certain distinctive characters of primary importance.

So within the realm of the last-named classes the phenomenon of ossification is wholly unknown. On the other hand, the ossification, as it has manifested itself in bony fishes, calling forth such bony pieces as the hyomandibular, the quadrate, the different pterygoids, the palatina, the maxillary

and premaxillary, the dentale, the angulare and the articulare, reveals itself by identical bony parts higher up in the scale of vertebrates. The general homology is even so close that we find no difficulty in comparing the elements of the skull and visceral arches of those bony fishes even in detail with the higher mammals and with man.

It will at all events be necessary to consider most carefully whether we had not better drop the primary division above noted of the Vertebrates into Anamnia and Amniota, as this subdivision has not even contributed to help us to understand the amnion better, and has on the contrary kept apart certain classes which earlier naturalists, as Linnæus and others, never thought of separating so widely. I expect that palæontologists will also contribute willingly to efface a separation based on a distinctive character which could never be applied to the objects of their research. Whereas at the same time the other anatomical differences between Reptilia and Amphibia, for example, break down in the case of very numerous fossil forms of very great importance.

Having up to now discussed the fœtal envelopes and appendages that are primarily of ectodermic origin, we have now to consider those in which the entoderm is the primary constituent. These are the umbilical vesicle and the allantois, of which the latter in many cases has actually assumed the shape of a vascularised embryonic envelope of respiratory or nutritive significance in Sauropsida and in many mammals.

2. The Umbilical Vesicle.

The umbilical vesicle in mammals may be grouped according to some few modifications, of which we will have to discuss the respective value and genesis. The first is that which we find in man, monkeys, and *Tarsius*. In these mammals the umbilical vesicle from the very first remains smaller than the trophoblast, never filling the whole of it. We have seen on p. 44 how the rest of the trophoblast comes to be lined by

ventral mesoblast at very early stages. On the surface of the umbilical vesicle of both man, monkeys, and *Tarsius* a very intricate net of bulky blood-vessels is developed. To a certain extent these blood-vessels may contribute to bring about exchanges between the fluids contained in the cavities both of the umbilical vesicle and the extra-embryonic cœlom outside of it (which is shut off from the exterior by the diplotrophoblast) and between the embryonic blood. It is, however, not known whether these fluids contain nutriment that might be of use to the developing embryo, as is the nutriment contained in the yolk-sac, say, of *Sauropsida*. Nor is it known whether the uterine lumen of *Tarsius* contains anything resembling uterine milk which could be transferred by a special action of the trophoblast cells inside the blastocyst, and from thence by the blood-vessels of the umbilical vesicle be transported towards the embryo. And also for the catarrhine monkeys are we yet in the dark as to whether the annular zone of non-proliferating trophoblast, which separates (see Fig. 132) the dorsal from the ventral placenta is utilised in the same direction, and whether in that case the vascular net of their umbilical vesicle would effectuate osmotic absorption and transportation of materials that had passed from the uterine lumen into the extra-embryonic cœlom of the blastocyst. For man and the anthropomorphæ such a process would be in any case excluded, their blastocyst being enclosed in a decidua reflexa, and not possessing the ring-like zone just mentioned for lower monkeys.

Under these circumstances considerable doubt must be entertained as to the efficiency of the vascular area of the umbilical vesicle for nutritive or respiratory purposes among the Primates. A suggestion which I published some years ago (Hubrecht, '99), and which has been independently brought forward by Saxer ('96) and Spee ('96) should then be considered more closely, viz. that this very fine-meshed net of considerable calibre has, in the first place, a hæmatopoietic significance.¹ There is no reason for wonder

¹ It should be borne in mind that the bone-marrow as focus of hæmato-

that the surface of the umbilical vesicle should, during the embryonic period, play an active part in this direction, if we remember how copiously blood formation is going on during embryonic life in another derivate of the entoderm, viz. the liver, not to mention the increased significance which we have to ascribe to the entoderm as the primary source of blood and blood-vessels, since the recent researches of Rückert and Mollier ('06). One of the main arguments of Ed. van Beneden ('99, p. 333) for not accepting my views on the phylogeny of the Mammalia was this, that the presence and the considerable development of the umbilical vesicle of the Mammalia could according to him only be explained, if for mammals we accept a reptilian ancestor with meroblastic, yolk-laden eggs. Van Beneden's reluctance might dwindle away if this hæmatopoietic significance of the surface of that part of the entoderm which has grown out into an embryonic appendage, and has been styled the umbilical vesicle, was found to be its chief "*raison d'être*."

That this hypothesis is not specially intended to stand as an argument against van Beneden's criticism follows from Spee's independent advocacy ('96) of the hæmatopoietic significance of this dense, vascular, network on the human (and simian) umbilical vesicle (Figs. 74, 156, 157).

The question once again arises if this significance of the vascular area on the umbilical vesicle of the higher Vertebrates is not older than that other property which it has assumed in the meroblastic eggs of Sauropsida, viz. the pro-

poietic processes is not yet present, and that also the liver cannot be said as yet to furnish a sufficient number of blood-corpuscles for assisting in the metabolic processes of the Primate embryo. And so the development and the increase of an extensive hæmatopoietic network on part of the intestinal surface (which from the very first had its significance as matrix for vasifactive mesenchyme) is quite natural. Thus, a hernia-like expansion of part of the gut would first have had a hæmatopoietic significance (Primates), would then have secondarily acquired significance in omphaloidean placentation (many mammals), and would finally have co-operated (Sauropsida) towards the transport of a reserve of yolk-substance, which in these cases had become accumulated against the inner surface of this network.

perty that these vessels of the area vasculosa, ramifying over the yolk, as they did before the entoderm cells have become yolk-laden to that extent, carry this reserve-food towards the embryo.

That the blood-vessels of the umbilical vesicle in mammals can attain a high calibre (Fig. 74) and that nevertheless the enclosed blood-cells have all the appearance in sections of not yet being freely suspended and movable I have noticed on earlier occasions ('89, '90). I have since found this confirmed in *Tarsius*, *Tupaja*, and others. And it would certainly not advocate against the hæmatopoietic significance of these blood-vessels, that also in Teleosts Wenckebach ('86) and Ziegler ('87) have shown that a solid chord may gradually develop into a vessel with a wide lumen and with blood-corpuscles that originally appeared as the inner core of the Anlage of the blood-vessel.

Having started from the consideration of the small umbilical vesicle of the Primates, we must now consider the case we find in the majority of mammals where the entoderm clothes the whole inner surface of the trophoblast at a very early period. So it does in Ungulates, but in these it becomes severed from the trophoblast comparatively soon again. This takes place when the mesoblast has developed and has become split into a somatic and a splanchnic layer. The splanchnic layer always remains very small as compared to the somatic in consequence of the enormous distension of the diplotrophoblast (Fig. 133). In other mammals, as in many Insectivores (Fig. 38), the separation between the umbilical vesicle and the diplotrophoblast is not so rapid, and sufficient time elapses before it comes about, so as to allow the vascular area that has in the meantime developed on the umbilical vesicle to become not only a centre of hæmatopoiesis, but now also a vehicle for a very appreciable exchange in a region which has been termed the omphaloidean placenta. I have elsewhere ('89, Pl. 18, fig. 32; Pl. 24, fig. 44) given a detailed description of this for the hedgehog and also for the shrew ('94 A, figs. 7—11, 51, 83).

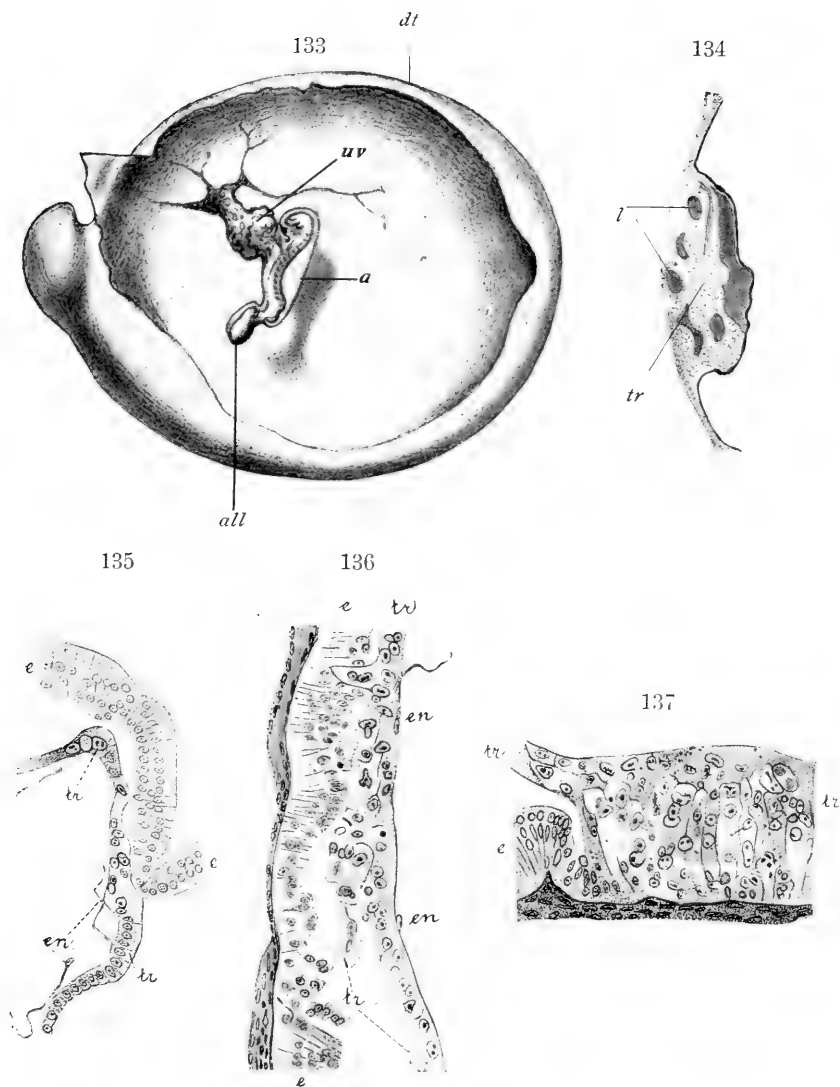


Fig. 133. The blastocyst of *Tragulus*, opened, to show the small umbilical vesicle *uv*, the amnion (*a*) and the incipient allantois *all*, *dt* diplotrophoblast (after Selenka, '91). — Fig. 134. Trophoblastic proliferations *tr* with lacanae (*l*) in which albuminiferous fluid is being absorbed (after Selenka, '87). — Fig. 135, 136 and 137. Sections through three stages of adhesion between uterine epithelium and trophoblast in *Tupaja* (after Hubrecht, '99). *tr* trophoblast; *en* entoderm; *e* uterine epithelium.

A strongly developed vascular network on the umbilical vesicle is also found in many Didelphia (Fig. 130), where the vascular area is undoubtedly in a high degree of nutritive significance during the short stay of the blastocyst in the uterus.

In the Ornithodelphia viviparity has been replaced by oviparity. There is no voluminous albumen layer, and the yolk-laden blastocyst is narrowly enclosed in the egg shell, a detail which renders the safe handling of the early embryonic shield exceedingly difficult. I believe this meroblastic arrangement in the Ornithodelphia to have been preceded by viviparity and by a relation of the spacious trophoblast to the formative ectoderm such as it was described and discussed above, the evolution of the allantois having come about during this viviparous phase. Perhaps we may yet regard the aberrant way in which the area vasculosa spreads over the yolk sac as a primitive character. Instead of being restricted to a circular region, the blood-vessels of *Echidna* invade the total surface of the umbilical vesicle (here: yolk sac) although they do not form so dense (Semon, '94, Figs. 61 o and 61 s) a network as they do in the Primates.

In the Sauropsida the phenomena of yolk-increase, and specialisation in the area vasculosa have varied in different directions.

3. The Allantois.

We now come to discuss the last of the embryonic appendages or foetal membranes, which are looked upon as characteristic for Sauropsida and Mammalia, viz. the allantois. This again was naturally first known in the chick, and what was revealed about it by this venerable archetype of vertebrate embryology was applied and adapted as best could be to the other higher vertebrates.

In the didelphic and monodelphic mammals its function could not be, as in birds, an extension against the egg-shell for respiratory purposes. But even in these it is seen soon

to spread out against that portion of the blastocyst-wall, where the placenta is going to be formed. Thus in mammals the allantois was both anatomically and physiologically the homologue of what was designated by that name in Sauropsida.

One difficulty was this, that in man no free allantois was ever detected, the (involuntary!) attempt of a German embryologist to let a chick-embryo stand for an early human foetus, only serving—once the fraud detected—to emphasize the existing difficulty. Moreover, further investigations showed that in no monkey, and not either in *Tarsius* spectrum was any free allantois present.

It seems to me that the confusion even now yet rampant concerning the phylogeny of the allantois would not have arisen if evolutionary principles had been more logically adhered to in the attempts to trace that phylogeny.

Observation shows that the main significance of the allantois in the developmental history of the higher vertebrates is a nutritive one, thanks to the strong vascularisation of its walls and the close contact into which these are brought either with vessels of the maternal mucosa (many Ungulates, Lemurs, and others) or with blood-spaces in the trophoblast, to which maternal blood gains admittance, thanks to the transitory arrangements furnished by the trophospongia (most other placental mammals).

Vascularisation of the diplotrophoblast or of the chorion (as I have proposed ['96, '99] to continue calling the outer embryonic envelope only in Primates) is thus the outcome of the developmental phenomena noticed in what has been called the allantois. And there is reason to believe that such mammals as have attained this aim most completely and at the earliest moment will be safer guides for teaching us how the arrangement may have come about phylogenetically than those in which the appearance of this vascularisation is for one reason or another retarded.

Now the Primates, who have no trace of omphaloidean placentation, but whose trophoblastic attachment to the

maternal mucosa in the region where the placenta will later appear is most precocious and elaborate, are decidedly in the first case.

And still there is here no free allantois at all. We must consequently try to analyse whether the method, according to which the vascularisation of the trophoblast comes about in the Primates, points in the direction of secondary changes by which the formation of a free allantois was precociously forestalled, or whether, on the contrary, the phenomena are such as to make it probable that the vascularisation is here brought about in a yet simpler, more direct, and more primitive way. In the latter case comparison with those mammals that possess a free allantois is none the less necessary, but then we may expect to meet a free allantois in its earliest incipient stages, only in the Primate stem in geological periods so far back that we can safely say that it will never actually reveal itself to us. These Primates, reaching back into the mesozoic and palæozoic epochs, had evidently better be called Proprimates, or even Protetrapods. As soon as a free allantois arose a step was taken in the direction of one of the numerous sidebranches: Sauropsida, Ornithodelphia, Monodelphia, etc.

At the same time we will then have to look out for transition forms which may serve to explain how, out of the more primitive arrangements of the Primates the free allantois of other mammals, and of the Sauropsida has come to evolve.

Now of the Primates that have no free allantois, man and the monkeys have not yet furnished a material of sufficient extension to study the very early stages of their vascular attachment in detail. For this we have to fall back upon *Tarsius*, which I have been able to investigate on this point ('02) in sufficient numbers to enable us to emit a constructive hypothesis with respect to what is called the "Haftstiel" or "Bauchstiel," i. e. the connective stalk by which the embryo communicates with the vascularised trophoblast, without any free allantois having preceded it.

And so we must just recapitulate what we notice in *Tarsius*. The very small blastocyst of about .03 mm. diameter has only just become attached to the maternal uterine epithelium. That portion of its trophoblast which serves for the attachment proliferates considerably, and enters into firm union with the correspondingly proliferating trophospongia (Hubrecht, '99, Figs. 13, 55, 56).

The blastocyst thus attached has only just passed through the phase (see p. 12) in which the trophoblast has opened out above the ectodermal shield (in a few cases I even found this process somewhat retarded: '02, Figs. 49, 50). This shield is by no means situated diametrically opposite the point of attachment (Fig. 62), but on the contrary so as to bring the hinder end of the ectodermal shield in the most immediate vicinity of the place of attachment to the maternal mucosa (Hubrecht, '07, Fig. *w.*, *w.*). From this hinder end of the ectodermal shield we have seen in a former chapter (p. 38) that the so-called ventral mesoderm proliferates backwards and downwards, at the same time becoming extended into a vesicle of extra-embryonic coelom. In this vesicle the direct and axial prolongation of the original starting-point of the proliferation is encountered as a raphe of tissue, a thickened ridge of only a few hundredths of a millimeter in length. This raphe is already in this very early phase the "connective stalk" by which the embryonic shield is in communication with the proliferating trophoblast that inaugurates the placenta. There is not the least reason to look upon it as an eventual precocious segregation of anything like a free allantois; it is early mesoblast, neither yet splanchnic nor somatic by which the embryo is from the very earliest connected with that portion of the surface that is going to be the placenta. There is as yet no question of its being vascularised. And it is the method by which this vascularisation is going to be brought about which will show us the way to an interpretation of the allantois, quite different from that contained in the text-books. At the same time more satisfactory, because it is an explanation of the phylogeny of

the allantois based on facts that are not only furnished by the higher but also by the lower vertebrates.

In studying the problem how the vascularisation of this early raphe or connective stalk of mesoblast is brought about we must bear in mind that in a former chapter (p. 33), we have established that the starting-point for the vascular system, as we find it outside and inside of the embryo, is an annular zone of entoderm, which, at an early stage of the development of *Tarsius*, lays the foundation both of the blood-vessels and of the blood.

We saw the endothelium of the heart derive from the entoderm, cells in the anterior portion of the protochordal plate ('02, Fig. 73, *a, b*), we saw the blood-vessels on the entodermal wall both in the intra- and in the extra-embryonic vascular regions take their origin out of the entoderm ('02, Fig. 59, *c-f*), as this was also observed for *Petromyzon* by Goette ('88, '90); for *Selachians*, by Swaen and Rückert; for *Teleostei*, by Swaen et Brachet ('99); for *Amphibia*, by Goette ('75) and Brachet ('02, '03); for birds, by Balfour and Deighton; for mammals (sheep, *Tupaja*, *Sorex*), by Bonnet ('84, '89) and myself ('90).

Moreover, the most intense manifestations of the production of blood-vessels in *Tarsius* is given in the hinder region, where the annular zone of mesenchyme-producing entoderm underlies the median zone of the ventral mesoblast ('02, Fig. 59, *g-k*). In earlier stages it is this posterior median zone ('02, Fig. 54, *g-k*) which commences vascularisation of the mesodermal raphe we are here discussing, thus laying the foundation of the blood-vessels in the "connective stalk." Now in this region of the stalk we can imagine the vasifactive phenomenon to have become exceptionally active if we remember that vascularising the "stalk" means at the same time the possibility of direct vascularisation of the diplo-trophoblast. This direct vascularisation would undoubtedly constitute so great an advantage to those mammals possessed of it (see pp. 34 and 102) that we can well imagine a vascular hypertrophy arising. Also in somewhat later stages

evident proof of this has been forthcoming ('02, Fig. 75, *i*; 77, *h—k*).

Now considering the two facts (*a*) that the first source of the vasifactive tissue is always the entoderm, and (*b*) that the stalk necessarily increases in length with the growth of the embryo (yet more so in *Tarsius* than in monkeys and man, because in *Tarsius* it bends round towards the surface¹ of the blastocyst opposite to the embryo), then we cannot wonder that active entodermal tissue is left behind in the stalk even when the rest of the intestinal wall undergoes the folding processes by which its definite tubular shape is gradually brought about. This entoderm, of which I have been able to follow even the very earliest apparition ('02, Figs. 56, 57, 59—61) takes the shape of a tubular extension in the lengthening stalk. I have particularly called attention to the fact that it does not grow into the stalk actively, but that it has spun out ('02, Fig. 11, *a—c*, Taf. XII) passively, as we saw was the case with the lengthening of the notochord (p. 37), and similarly with the thickening of the placenta (p. 125).

It is not suggested here that, when once the connective stalk is thoroughly vascularised and the vessels carried by it have spread out on the inner surface of the placenta and have provided branching capillaries for the vascularisation of the embryonic placental villi, any further vasifactive processes go on or start from the endodermic epithelial tube,

¹ This expression should be understood *cum grano salis*. It is not the stalk that bends down, or has during its lengthening process bent down towards that opposite surface, but it is the embryonic shield that has, so to say, crept upwards (as I have elsewhere described ['02, p. 19; '07, Figs. *w*.^{1—w}.³]) along that surface of the blastocyst, which is opposite to the placental attachment (Figs. 62—65). The shield region is originally situated quite close to the surface of attachment; later, when the amnionfolds are being formed, it is found diametrically opposite to the latter. This change in the situation of the embryo with respect to the placenta does not occur in monkeys and man, hence the connecting stalk in their case is much shorter than in *Tarsius*. At the same time they have for this reason their backs turned towards the placenta, *Tarsius*, on the contrary, its ventral surface.

which represents in this stalk what will in other mammals and in the Sauropsida be the inner cavity of the allantois. This epithelium is only a remnant of what has in earlier stages been a proliferating vasifactive region of the endoderm, and which, after being yet active for some time ('02, Fig. 61, 65, 66), finally lapses into the position of a residuary structure ending blindly and being in the umbilical cord of the later foetal stages only difficultly recognisable as a string of cells of quite a rudimentary character.

We have now followed the connective stalk of *Tarsius* and the endodermic epithelial tube within it, which we will call allantois, in its entire development. It is, in the present state of our knowledge, no unfair assumption to say that the genesis of the connective stalk of man and monkeys, with its epithelial endoderm tube, which there also is designated as allantois, must correspond in its principal characteristics with what we have just described. And we may now emphatically affirm that the Primates have no free allantois, but that the stalk-like connection between embryonic shield and embryonic envelope is of so early a nature, and can be so perfectly explained without having recourse to any endodermic outgrowth, that we are justified in looking upon this peculiar arrangement of the connective stalk of the Primates as more primitive than the free allantois of any other of the higher Vertebrata.

Now let us for a moment try to realise how those who take the free allantois as the more primitive have to picture for themselves the phylogenetic origin of it. I have elsewhere ('07, p. 58), in discussing this question, expressed myself as follows:—"We could not possibly imagine that the allantois arose as an independent vesicle spontaneously growing out from the hind gut. At what stage of phylogenesis would this have been inaugurated? Has ever any amphibian-like animal been struck by the happy thought that it might allow its urinary bladder to undergo a so much earlier development, in order that it might obtain a so much more considerable size and such a copious vascularisation? And that in this

way that most important larval organ, the allantois, all of a sudden originated, the organ by which nutrition and respiration is brought about, and which has become reduced in man, the monkeys, and *Tarsius* to the connective stalk ? ”

I doubt whether any embryologist will be found willing to adhere to this conception.

I continue to cite from the Normentafel publication ('07, p. 58) :—“ We may yet further point out that the very latest and very thorough going investigation of Peter ('05) describes and figures certain (Taf. I, figs. 9–11 ; II, figs. 14–18) details which also Strahl ('81, '82, '83) and Corning ('95, Figs. 4, 7, 9) had noticed before. According to these investigations, the allantois of *Lacerta* originates so very early as a solid *Anlage* in the hinder axis of the embryo (the cavity appearing only later, and yet later opening out into the gut) that the respective relations could not have been different if the allantois of *Lacerta*, instead of being derived from a free outgrowth of the gut, had on the contrary evolved out of an earlier and solid connective stalk in the axis of the embryo.

The relations between the allantois of *Tarsius* and *Nycticebus* are further yet illustrated by Figs. z^{1-3} and aa^{1-3} of the Normentafel ('07). From them we learn that what is called the allantois of *Tarsius* belongs to the very oldest parts of the gut, and that the caudal gut (Schwanzdarm) only originates later as a protrusion directed dorsally. If we consider how matters stand in *Nycticebus* 92, 148 and 239 ('07, Tabelle 2, 3, and 4), then we see that the hinder elongation of the gut, which we notice in the caudal extremity above the umbilical vesicle, might be considered to resemble remains of a connective stalk as much as anything else. The ventral portions are already strongly vascularised in *Nycticebus* 92, more yet in 148, and it can, for 239, be just as well said that the caudal gut develops, as in *Tarsius*, as a dorsal protrusion from the posterior (connective stalk) portion of the gut, as that one should conform to the current view and look upon the protruding allantois as a free vesicle

which has been later evolved.¹ And yet it is also in *Nycticebus* that out of this early primordium the comparatively spacious allantois originates, which spreads against the diplotrophoblast in the well-known way. However, nothing would prevent us, neither in *Nycticebus* nor in *Lacerta* mentioned before, to look upon the particular features of the formation of their allantois as so many reminiscences of an earlier connective stalk.

I have reason to believe that many hesitate to accept my conclusions concerning these embryonic phenomena, because for them the derivation of the mammalian blastocyst out of a yolk-laden sauropsidan egg, as it has since a long series of years been taught in all manuals of embryology, seems too well established, all the more so because the *Ornithodelphia* appear to be such typical transitions.

It is, however, my conviction that in the *Ornithodelphia*—as in the *Sauropsida*—a profusion of yolk and oviparity have both arisen only after viviparous ancestral forms had preceded, in which a larval envelope (trophoblast) and its respective derivatives (diplotrophoblast, amnion) were already present. Rapid vascularisation of the trophoblast by means of umbilical vessels (as it must have existed in those ancestors) was replaced in those descendants that obtained a considerable increase of yolk by an early vascularisation of the wall of the yolk-sac (*area vasculosa*). Only later the palingenetic vascularisation of the larval envelope (trophoblast) again comes to

¹ It is certainly striking that both from Corning's figures ('95) of *Lacerta* and from those of Bonnet ('84, '89) of the sheep, it follows that also, according to these authors, the allantois appears earlier than the caudal gut; and that thus the conception of the allantois as the older, posterior, axially situated elongation of the intestine (cf. Hubrecht, '02, xv, figs. 5 and 7), that has acquired great importance for the vascularisation of the equally primitive connective stalk, appears quite valid. Moreover, by this conception the phylogeny and the ontogeny of the allantois are more easily reconciled than by that other, which sees in the allantois only a later vesicular formation which protrudes *ad hoc*.

the front and co-operates to bring about favourable conditions for respiration."

If we try to find out whether among living mammals there are such that would yet exemplify transitional stages, as they must have existed between such ancestral forms that possessed (as do yet the Primates) a more primitive "connective stalk" and such that have come to evolve a free allantois, we must conclude that such forms are few. However, where they are found—among the Rodents and in *Galeopithecus*—we must recognise that we have one of the lower orders before us. In the Insectivores, where we might have expected to find traces, those that have hitherto been examined all possess a free allantois, but then they vary so considerably among themselves that we have reason to look forward hopefully to those Insectivores whose ontogeny has not yet been traced.

Among Rodents are *Cavia* and the different mouse genera where the allantois offers peculiarities that might here be adduced. These cases are, however, the same in which the phenomenon of the so-called inversion of the germinal layers occurs, and one might be doubtful as to whether this latter phenomenon were not rather responsible for the peculiarities of the allantois. However, I have attempted (on pp. 74, 75) to connect this phenomenon with primitive features also noticed in the development of invertebrates. We must for the present suspend our judgment and recognise that the whole process of inversion has still much that is obscure and can on no account be explained by apparently simple mechanical explanations as Selenka has attempted ('84, p. 70). An early circumscribed attachment of the blastocyst to the maternal uterine wall was in his estimation the cause which rationally explained that the embryonic shield becomes bent upon itself (entypie). He neglected to consider that in a case of very early "entypie" as it is found in *Tupaja* and was fully discussed above (p. 10, Figs. 29—32), the blastocyst is during all these phases perfectly free and unattached in the much wider lumen.

On the other hand an embryonic shield, if curved in this way and then developing an early ventral mesoblast by proliferating ectoderm at the hinder end of the embryonic shield (cf. p. 92), would be very favourably situated for an early vascularisation of the trophoblast, all the more so if a decidua reflexa—as is also met with in many Rodents—co-operated. And so it would not seem impossible to turn the argument the other way, and to pretend that the very inversion was in some way connected with an earlier presence of a vascular “connective stalk.” I do not, however, wish to press this argument, as I feel the ground is as yet too uncertain. I have only wished to call attention to those rodents and eventual insectivores where the first rudiment of the allantois is in no way an outgrowth from the hind wall of the gut, but simply a proliferation in a very early stage of vasifactive tissue at the hinder end of the embryonic shield. I may call attention to the fact that very many years ago I have already suggested ('89, p. 375) that potentially the possibility of the development of a connective stalk was present in the hedgehog, and was only perhaps retarded by the fact of the omphaloidean placentation having acquired considerable significance in early stages.

Summarising we may say that the allantois, such as we find it in Sauropsida and in many mammals and which has erroneously been looked upon as being primarily a urine-reservoir during embryonic life, is not the fit starting-point for phylogenetic speculations about its evolution, but that the more subordinate position in which we find it in the Primates offers a clue for the explanation of its earliest appearance. At the same time it makes us understand so much better, that the favourable conditions under which the Primates succeed in establishing an early and very thorough vascularisation of their trophoblast, has contributed largely to their exceptional development in regard to the central nervous system.

We can safely say, as I wrote recently ('07, p. 60), that the different orders of mammals represent so many attempts

by which nature, starting from simple methods of vascularisation of the external embryonic membrane, has tended to create a very wide range of adaptation to all the different possibilities of nutrition which are offered to the embryo. The uncommon diversity which we observe in the endless varieties in the arrangement of the foetal membranes of the mammals would be as good as inexplicable if we held on to the derivation of the monodelphian mammals out of yolk-laden ancestors with ornithodelphian characters. And since, after Hill's ('97) investigations, we must assume that the didelphian mammals are not descended from Ornithodelphia but from monodelphian, placental ancestors, they no longer form an imaginary transition between Ornithodelphia and Monodelphia.

Thus a more direct phylogeny of the latter should form the object of diligent research, towards which the present paper is a first attempt.

CHAPTER IV.—THE PART PLAYED BY THE TROPHOBLAST IN THE NUTRITION AND THE ATTACHMENT OF THE EMBRYO.

In Chapter II we have discussed the trophoblast as a larval layer which is of great importance in monodelphian and didelphian mammals, but which, in the Sauropsida, has diminished both in importance and in distinctness parallel to the development of oviparity, and of which we may presume that even among lower vertebrates rudiments are yet retained.

Suggestions have been thrown out on p. 18 that the original significance (protective, locomotor, or otherwise) of the ancestral larval layer may have gradually become converted into an adhesive and a nutritive one. For this I want in this chapter to adduce all the evidence available.

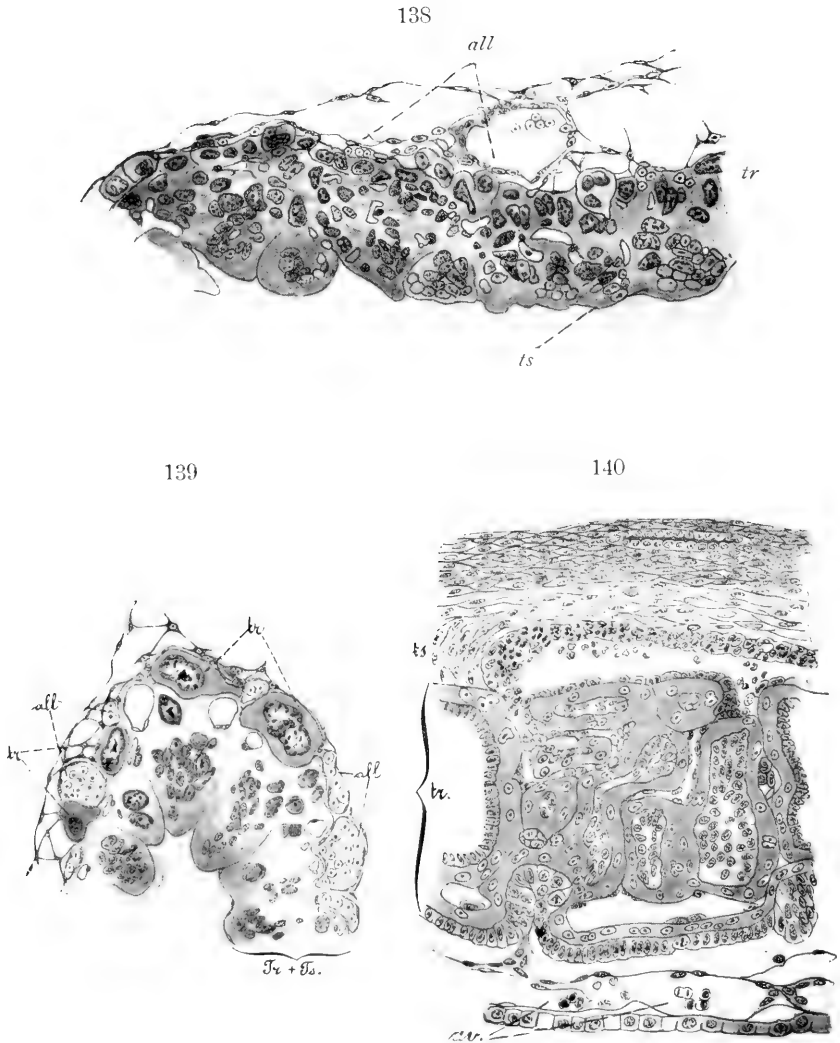


Fig. 138 and 139. Two sections through two successive phases of placenta-
 tion of *Perameles*. *all* allantoic vessels, *tr* trophoblast, *ts* maternal tropho-
 spongia (after Hill, '97). — Fig. 140. Section through the placenta of the bat
 (after Nolf, '96). *ts* maternal trophospongia. *tr* embryonic trophoblast, yet
 including remains of the endothelia of maternal capillaria which in other
 places have disappeared by resorption. *av* allantoic vessels with embryonic
 bloodcorpuscles.

1. Didelphia Nonplacentalia.

An important case is that of the opossum, in which we notice in Selenka's figure here copied (Fig. 134) how a considerable proliferation occurs in the trophoblast at a yet very early age, and how in this proliferation cavities or sinuses appear in which the surrounding nutritive fluids contained in the uterus lumen and partly enclosing the egg as a sort of albumen layer can penetrate. There is no doubt that this nutritive matter, when once it is surrounded by trophoblast cells, many of which undergo special proliferation, can be absorbed in an accelerated and intensified fashion, and is then utilised for the benefit of the developing embryo, most probably by its passing in some form or other inside the cavity of the umbilical vesicle.

We have not to go higher than these same Didelphia to find that also properties of adhesiveness are characteristic for the trophoblast cells. In the genus *Perameles* it has been made known by J. P. Hill ('97) that far from being aplacental—as was the current opinion concerning all Didelphia—the blastocyst of this genus possesses a very well developed adhesive surface, by which it fuses with the maternal uterine mucosa, and against which after a time allantoidean blood-vessels become applied, thus forming a full-fledged allantoidean placentation.

The trophoblast at these points of adhesion between blastocyst and maternal epithelium undergoes marked changes, as can be concluded from Hill's Figs. 138 and 139. About the nature of the fusion between maternal epithelium and trophoblast we will have something to say further on (p. 115)¹; here it may suffice to state that the changes are only brought about in those trophoblast cells which partake in the placentation process, not in those which are present over the

¹ I may here add that I differ from Hill in the interpretation of the later stages of the *Perameles* placenta, and that I am inclined to ascribe a much more considerable part to the proliferating trophoblast than he does.

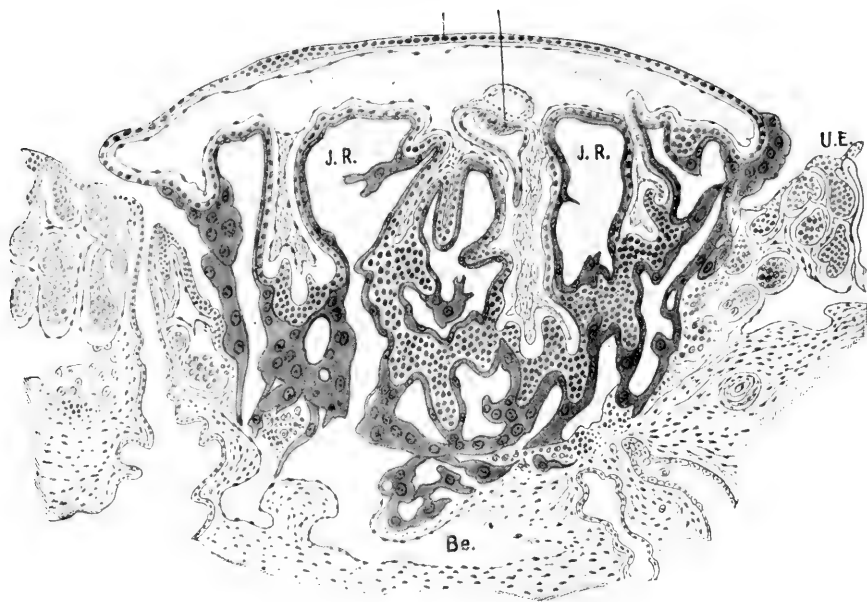
remaining surface of the blastocyst, which does not adhere to the maternal mucosa.

A second most instructive case of attachment of the didelphian blastocyst to the maternal tissue we also owe to Hill when he described the early stages of *Dasyurus* ('00). He finds the allantoidean diplotrophoblast in full retreat and degeneration, the allantois itself hardly vascular and evidently abdicating. The contact with the maternal nutritive matter is brought about during the eight days of gestation by a ring-shaped zone (*av*, Fig. 130) where the omphaloidean vessels form a vascular ring, which undoubtedly facilitates the respiration of the embryo, while below this ring another ring of peculiarly developing trophoblast constitutes still another surface upon which nutritory processes are inaugurated (*atr*, Fig. 130). This lower ring is about one and a half the width of the vascularised omphaloidean ring, and is also distinguished from the latter by the much more considerable activity of the trophoblast cells that form the outer layer of this omphaloidean diplotrophoblast. Hill describes in detail how the trophoblast cells surround and destroy part of the maternal uterine epithelium, how they then reach maternal sub-epithelial capillaries, how they envelope these and gradually develop into a syncytium of undoubted nutritive significance for the embryo. It is interesting to note that at birth the embryonic proliferations are not shed (nor is any maternal tissue), but that they are absorbed *in situ*, as I have described it for the mole (contradeciduate type of placentation, p. 124).

2. Monodelphia.

Passing on to the monodelphian mammals, we find an endless variety in the adaptation of the trophoblast to early phenomena of adhesion, of nutrition, and of phagocytosis, the latter leading up to an actual embedding of the blastocyst in maternal tissue, thus ensuring an all the more extensive possibility of mutual osmotic interchange between the maternal and embryonic vascular systems.

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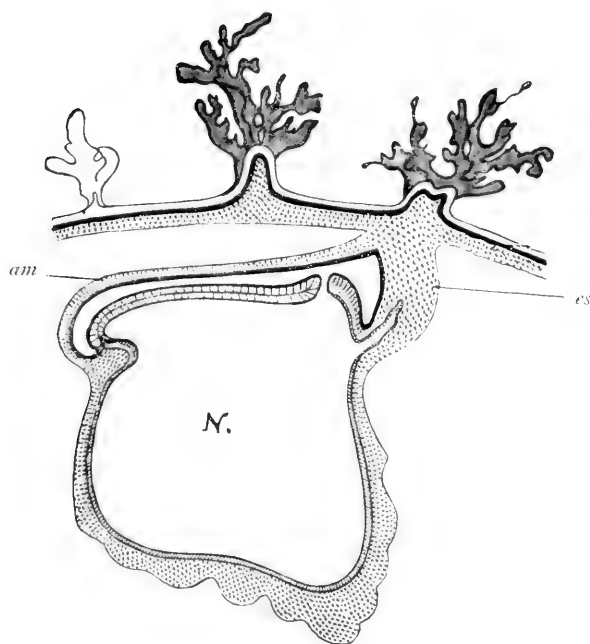


Fig. 141. Longitudinal section through an early human blastocyst with amnion (*am*) neurorenteric canal, connective stalk (*es*) and allantoic tube (after Spee). Vascularized trophoblastic wall of blastocyst with villi only partially represented. *N'* umbilical vesicle. — Fig. 142.

Diagrammatic section through the blastocyst and early placental attachment of a catarrhine monkey (after Selenka, '00). *UE* uterine epithelium, *Ec* vessels in maternal trophospongia, *JR* lacunae in trophoblastic tissue, filled with maternal blood.

Between the extremes such as we find them on one hand in the Ungulates, where the young blastocyst undergoes an immense increase in size before the processes described in Chapter III are inaugurated, and on the other hand in certain Primates, Insectivora, and Rodentia, where the blastocyst is yet uncommonly small when these processes are started, every possible gradation has already been observed in different orders of Monodelphia. It may in general be said that in the first-named case, when there is an enormous initial increase in surface, the changes which the trophoblast undergoes and the proliferations to which some of its cells are subject are much less considerable, whereas in the second case those changes and proliferations are ever so much more intense.

I have no hesitation in saying that the new functions to which the trophoblast must have become adapted, simultaneously with the gradual development of the amphibious Protetrapod ancestors into monodelphian mammals, were—each in its special significance—of the utmost importance for the different lines along which this development could proceed. The highest degree of development has been reached by those descendants whose trophoblast exhibits a maximum of activity, and in whom we at the same time find a maximum of useful adaptations in the blastocyst, by which the latter can have the full profit of the advantages thus offered by the proliferating trophoblast. This combination is not always present. Thus we find among primitive monodelphia the hedgehog and *Gymnura* with a very intense proliferation of the trophoblast (Figs. 36—38), but deficient in the way in which the blastocyst responds to and utilises the facilities thus offered. On the contrary, in man and the *Anthropomorphæ* the trophoblastic preparations resemble very closely to what the hedgehog shows us, but here the development of the blastocyst itself has followed a totally different line, and has reached a degree of early completeness and differentiation (Figs. 39, 40, 141, 143, 144), which secures to the developing embryo, during pregnancy, a combination of the most favour-

able circumstances as far as the conditions of nutrition are concerned. It cannot well be doubted that this has been very conducive to allowing the central nervous system to reach that stage of higher development and complication by which the human brain is so widely separated from that of other mammals.¹

More than once the mammalian blastocyst, when it actively attacks the maternal tissues, reminds one of a temporary internal parasite. It is clear that the more perfect the arrangements are by which the temporary parasite obtains its food from the mother-host, the more intense and perfected can be its nutrition and growth during life in utero.

a. Hedgehog (*Erinaceus*).—We will now select a few examples out of the numerous cases offered by all the different orders of mammals.

We begin by what may at the same time be looked upon as a primitive, as a full-fledged and a very instructive case, viz., the hedgehog, in which we find the above-said resemblance with the higher Primates, as was admitted by investigators of early human blastocysts, such as Siegenbeek van Heukelom ('98), and Peters ('99).

The blastocyst, at a period when the entoderm is not yet clearly separated from the embryonic knob (cf. p. 8), and when the cavity inside of the trophoblast is not either very spacious, is found in the lumen of the uterus at the bottom of a comparatively deep pit which has originated preparatory to pregnancy by a proliferation of the maternal tissue, which was fully described elsewhere by myself ('89, p. 312), and by Resink ('02). We find the trophoblast of the very young embryo closely applied against the maternal epithelium of the pit; then against the denuded subepithelial

¹ In connection with this I may just call in mind the curious fact that in other mammals, who, beside man, share those favourable adaptations of the blastocyst (*Tarsius* being the best known of them), a very unexpected increase in the volume of the central nervous system in its very earliest stages is noted. This increase has been more fully described and figured by me elsewhere ('07, p. 50, figs. *i*—*p*).

mucosa, the maternal epithelium being eroded just where the trophoblast is in contact with it, and finally becoming embedded in this mucosa, the mouth of the pit in the maternal tissue being at the same time closed by extravasating blood and by cell proliferation. There can be no doubt that in these three early stages the action of the trophoblast cells, both towards the maternal epithelial and subepithelial tissues, is strongly corrosive (perhaps chemically) or phagocytic (cf. '89, Pl. 22*a*, 23, figs. 39*a*, 41). Nor that in the now following stages (during which the trophoblast shows a very extensive proliferation all around the whole blastocyst) it eats its way yet further into the tissues of the maternal trophospongia,¹ and locally destroys the endothelium of fine capillaries which then shed the blood they contain into the lacunar spaces of this trophoblastic proliferation. These lacunar spaces are not of an irregular sponge-like shape. When seen in transverse section they are disposed (as cup-shaped arcades filled with maternal blood) round the internal cavity contained in the blastocyst (Figs. 36 and 37), which, as soon as the entoderm has come to line the inner surface of the trophoblast, will have become the cavity of the umbilical vesicle. Into this cavity the maternal blood which circulates in the lacunæ of the trophoblast can now with great facility give off such substances as may be selected by the trophoblast cells that form the separating wall between the maternal blood and the cavity of the umbilical vesicle. Between the contents of this cavity and the trophoblast cells there is only a thin layer of endoderm cells. Later the extra embryonic vascular

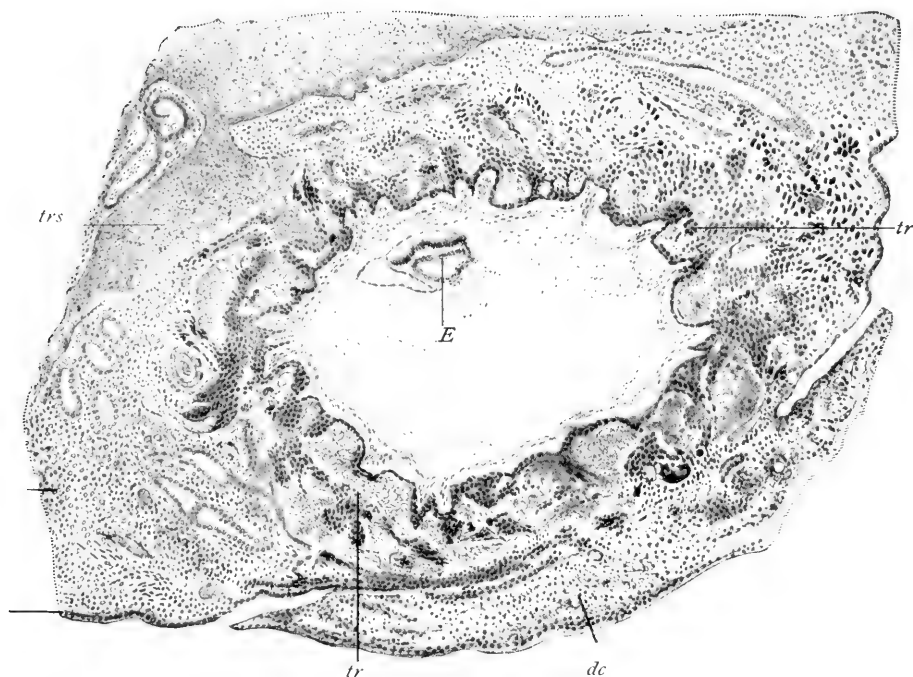
¹ The name trophospongia, which I introduced nineteen years ago ('89), is here taken in the sense in which I have used it since 1899 ('99, p. 350), and in which my pupil Resink ('02) has applied it to the hedgehog. It indicates maternal cell-proliferation, specially intended for the fixation of the blastocyst, and shows a different histological evolution in different genera (Sorex, Lepus, Tupaja, Tarsius, etc.). In the hedgehog I formerly called this proliferation the decidual swelling ('89, p. 311). For the hedgehog the amount of foetal trophoblast is thus seen to be yet more considerable than I dared to suppose originally, when I mistook part of the embryonic trophoblast for maternal trophospongia.

network (area vasculosa) of the umbilical vesicle will come to develop on this very surface. Conditions will thus arise that are yet more favourable for an interchange between the maternal blood, slowly circulating in the trophoblastic lacunæ and the embryonic blood-corpuscles winding their way along the paths of this area vasculosa of the hedgehog (Fig. 38). And later yet at another spot of this massive trophoblastic spongework, soaked with circulating maternal blood, the allantois of the hedgehog will find occasion to attach itself and to lay the foundation of the allantoidean placentation by which the earlier but provisional omphaloidean placentation (as the region of osmotic interchange above noticed is sometimes called) is succeeded. This will be further discussed in Chapter VI.

During the very considerable proliferation of the trophoblast in the hedgehog here alluded to,¹ a further specialisation of the different parts of it becomes apparent very soon. The outer layer comes to produce very large cells with big nuclei which in a former publication ('89, p. 325) I have termed deciduofracts, and which seem to have a further phagocytic effect on the surrounding maternal tissues. Up to now the hedgehog's trophoblast has retained the character of a massive spherical outer layer of the blastocyst, which is surrounded on all sides by maternal tissue (decidua capsularis), thanks to the closure of the mouth of the deep pit into which it has found its way in the earlier stages (p. 102). As development proceeds this capsularis thins out consider-

¹ I must here call attention to the fact that the trophoblast—considered by me in its earliest one layered stage as a larval envelope, inherited from invertebrate ancestors—remains morphologically perfectly equivalent to itself in later stages, however considerable may be the proliferation (and thus the increase in thickness combined with specialisation) which it exhibits at one or more spots, or even (as in the hedgehog and in man) over the whole of its surface. This should withhold us from applying the name of trophoderm to those proliferating portions of it, as Sedgwick Minot ('03) invites us to do. The name suggests a morphological difference which does not exist. For the purpose which Minot has in view, the older name of ectoplacenta proposed by Duval seems quite suitable (see p. 15).

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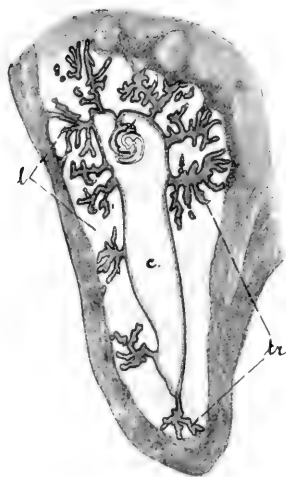


Fig. 143. Section through a very early human blastocyst imbedded in the maternal mucosa (after Peters). *tr* trophoblast, *trs* maternal trophospongia, *dc* decidua capsularis, *E* Embryo. — Fig. 144. The same of *Hylobates* (after Selenka, '99). *tr* vascularized villi with trophoblastic outer layer, *l* lacunae, *c* extra-embryonic coelom.

ably on one side, viz., the surface that is contiguous with the uterus lumen. This thinning out goes parallel to, and is largely caused by, the increase in size of the growing blastocyst. The natural consequence is, that also the trophoblastic investment of the blastocyst is simultaneously considerably flattened as far as it is covered by the decidua capsularis. The maternal investment retains its thickness only along a saucer-shape zone furthest from the original uterus lumen. It is in this part of the trophoblastic proliferation that the allantoidean placentation comes about and reaches its maximal development; a saucer- or disc-shaped placenta, which I have fully described in a former publication ('89), is the final outcome.

b. Primates.—The investigations of Peters ('99), Siegenbeek van Heukelom ('98), Selenka ('00), Strahl ('02, '04), Spee ('96), Kollmann ('92), and, quite lately, Bryce and Teacher ('08) have revealed to us that the early trophoblast of man and the anthropoid monkeys is very similar (Figs. 39, 40, 143, 144) in its general line of development to that of the hedgehog. The very early stages and the exact way in which the human blastocyst comes to be imbedded in the maternal mucosa are, however, yet insufficiently known. Moreover, as we will see in Chapter VI, the placentary lacunæ are more spacious and the villi partially free and floatingly suspended in the maternal blood that circulates in these trophoblastic lacunæ (Figs. 141, 144).

In the catarrhine monkeys, which differ from the Anthropomorphæ and from man by the absence of a decidua reflexa (capsularis), the trophoblastic proliferation is no longer equally distributed over the whole surface, but is restricted to two regions opposite to each other, and corresponding to what will later become the dorsal and the ventral placenta (Figs. 132, 142). There is thus a circular zone along which there is hardly any proliferation of the trophoblast. I hold this arrangement to be secondarily derived from the complete enclosure present in man and the anthropoids. Again in *Tarsius*, which I have also classed with the Primates on the

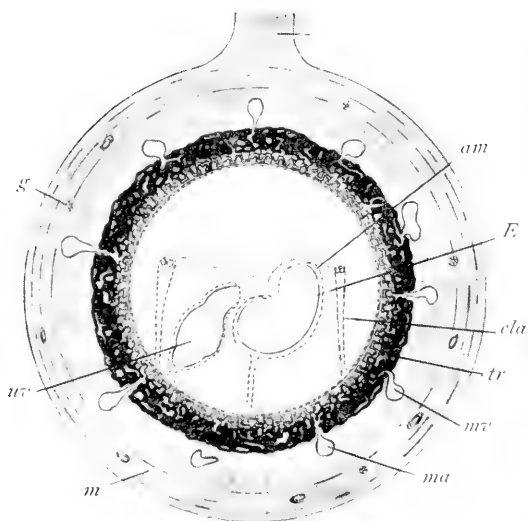
very pressing grounds which have already been discussed in preceding chapters, the proliferation of the trophoblast only takes place on a restricted portion of the spherical surface of the trophoblast, as I have demonstrated elsewhere ('94 B, '96, '99). It is by this restricted portion that the blastocyst first adheres to the maternal uterine epithelium, and it is here that proliferations arise which become fused with other proliferations of the maternal trophospongia in a way analogous to what we noticed for the hedgehog. Thus a vascular spongework is brought about, against which the developing placenta comes to be attached (Figs. 147, 150). The details of the trophoblastic differentiation of *Tarsius* I have described in my former paper ('99); it may here suffice to say that besides the development of lacunæ and large giant-cells with very peculiar nuclei, I have noticed an interesting phenomenon in these trophoblastic and also in maternal trophospongian cells.

This phenomenon, which will have to be inquired into also in other mammals, consists in the production of red blood-corpuscles out of these cells, or rather out of the nuclear matter, which undergoes a series of most remarkable changes and transformations. Those red blood-corpuscles, devoid of a nucleus, would thus be derived from nuclear matter of certain trophoblast cells. This is certainly less astonishing, since it has been shown by me in a former paper ('99) that the definite red blood-corpuscles of the embryo also arise by nuclear transformation in the nucleated blood-mother-cells.

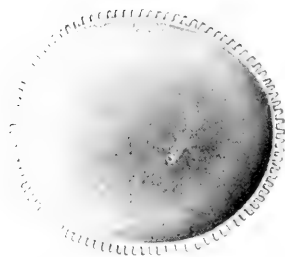
The production of blood-corpuscles by the cells of a larval envelope is surely an unexpected histological phenomenon. Still, the details of differential segregation during the successive stages of cell lineage are not yet well enough known to justify any apodictic negation. The possibility is not excluded that at the first cleavage (suppose this to separate trophoblast from embryonic knob) certain potentialities of hæmatogenesis may be passed on to this trophoblast mother-cell.

Besides the cases of trophoblastic proliferation, preparatory

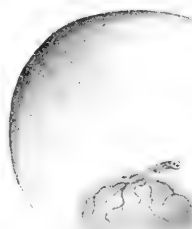
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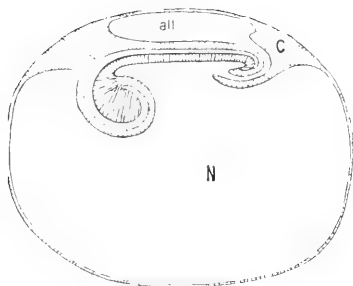
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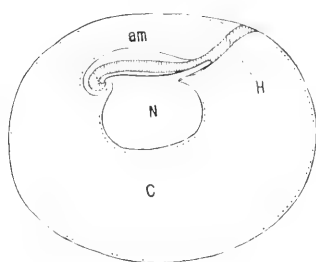
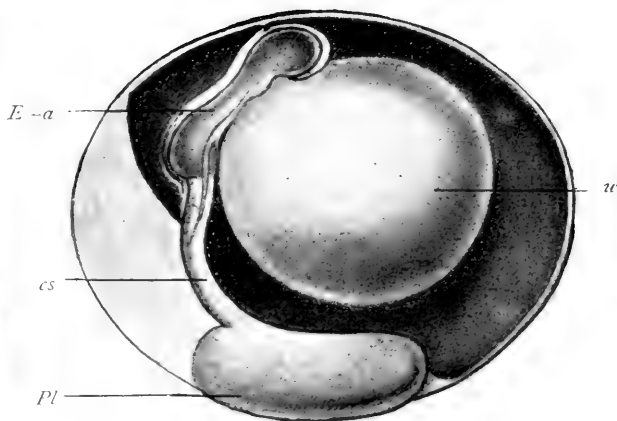


Fig. 145. Transverse section through uterus and foetus of Hyrax. *m* muscularis, *g* uterine gland, *ma mv* maternal uterine artery and vein, *tr* trophoblast with bloodlacunae and surrounding allantoic villi; *cla* entodermal lining of allantois; *E* embryo; *am* amnion. — Fig. 146. The allantoidean diplotrophoblast of *Nycticebus* looked into after removal of the embryo and of the left half. — Fig. 147. The non-vascular diplotrophoblast of *Tarsius* looked into after removal of the embryo. The presence of a connective stalk has led up to the formation of a discoidal placenta. — Fig. 148. Diagram of *Nycticebus* during the formation of allantoidean diplotrophoblast. *c* extraembryonic coelom. — Fig. 149. The same for the Primates, where the trophoblast is directly vascularised by means of the connective stalk *H*.

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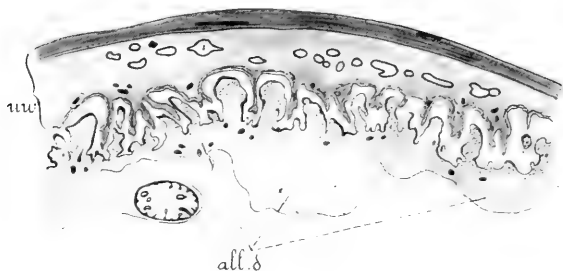


Fig. 150. Side view of reconstruction of blastocyst of *Tarsius* with placenta *pl*, connective stalk *cs*, amnion *a*, allantois and umbilical vesicle *uv* (after Hubrecht, '96). — Fig. 151. Embryo of *Chiromys madagascariensis* yet for the greater part enveloped in its allantoidean diptrophoblast, showing exactly the same features of placental arrangements as other Lemurs (*Nycticebus*, *Galago*). Original specimen in the British Museum. — Fig. 152. *Nycticebus tardigradus*. Transverse section of uterine wall (*uw*) with honey-combed inner surface against which the trophoblastic and vascularized embryonic villi fit. *all* allantoic tissue vascularizing the villi (after Hubrecht, '94^b).

to placentation, which have here been alluded to, we find the same in very diverse form among Insectivora, Rodentia, Carnivora, and others, and it would lead us too far to give a detailed account of all the possible variations known up to now. We can already conclude from a comparison of the hedgehog and certain Primates that the trophoblast's specific modifications are all the more considerable the more extensive the surface is over which the blastocyst comes into contact with and fixes itself in the maternal tissue. Tarsius, which had a comparatively limited surface of attachment, retains an unmodified trophoblast over a very considerable portion of the growing blastocyst.

c. Rodentia and Carnivora.—Among Rodents we similarly notice that besides cases in which the rapidly-enlarging blastocyst is only very partially attached to the mucosa (as is, for example, the case along a hoof-shaped part of the surface close to the embryonic shield in the rabbit), there are others in which the (generally comparatively much smaller) blastocysts disappear partially or wholly in the maternal tissue, and become in the latter case enclosed in a decidua capsularis, there being valid grounds for looking upon this latter process as the more primitive. The mouse, Arvicola, the guinea-pig are examples of this. Selenka ('83, '84), Duval ('87), Jenkinson ('02), Disse ('06), and others have given detailed descriptions of the very considerable modifications which the trophoblast cells undergo after the blastocyst has become definitely lodged in the maternal subepithelial tissue. They increase very considerably in size, become confluent for the formation of a syncytium, contribute towards the formation of spacious lacunæ for the reception of maternal blood in the immediate vicinity of the developing blastocyst; in short, they are of great significance for the welfare of the young embryo. In many Rodents the trophoblastic proliferation assumes a different character according to the part of the surface which we examine, F. Muller ('07), and in those where the embryo becomes wholly surrounded by maternal tissue the trophoblast does not necessarily behave as it does in the hedgehog,

where its proliferation is equally strong all over the surface. On the contrary, in the mouse, in *Arvicola*, *Cavia*, and others there is a very marked centre of proliferation, which will afterwards become the placental attachment, and which already, in the very early stages, consists of an accumulation of trophoblast cells to which Selenka has given the name of Träger (supporter) (Figs. 24—28).

The phenomena recorded in this chapter are not considered in the same light by all authors. Notably Strahl's interpretations contained in his extensive researches on this subject ('89 to '92) and in his chapter on mammalian placentation to Hertwig's *Handbuch*, differ considerably from my own. He is inclined to ascribe a much more considerable significance to the part which maternal tissue plays in the full-grown placenta. Many of the trophoblastic proliferations described in this chapter are by him considered to be of maternal origin. The latest author, however, who has thoroughly investigated the subject and who has published a very lucid exposition of his results, Schoenfeld ('03), adopts my views (l. c., p. 814), and differs both from Strahl and from Bonnet ('97-'01). The latter, though also studying the dog, as did Schoenfeld, has probably declined to accept the possibility ("le fait pouvant paraître bizarre" Schoenfeld) of the existence of a mixed plasmodium in which both foetal and maternal elements are represented. Such a plasmodium was detected by myself in *Tarsius* ('99, Figs. 62—64) and *Tupaja* ('99, Figs. 51—54), by Schoenfeld in the dog (l. c., Pl. 24, fig. 6), and enabled the latter author to establish the real nature of the placenta of the Carnivora, towards the interpretation of which the views of Duval ('94, '95) and Strahl ('90A, '94) presented conflicting interpretations.

I may add that Schoenfeld's results according with and confirming those which I had obtained in *Insectivores* and *Primates* (and equally applicable to the rabbit, which was also personally investigated by Schoenfeld) seem to me to open up a line of research by which we will be able better to understand the placentation of those *Ungulates* and *Lemurs*,

in which, as was hinted at above, we might be tempted to deny the presence of any placenta, and which yet, for several reasons, I do not consider as primitive in respect to placentation. The so-called diffuse placenta has been looked upon by Strahl and the older authors as the necessary starting-point from which more complicated and more specialised systems of placentation should be derived. In this they were wrong. The presence of this diffuse placentation in such orders as Lemures, Cetacea, Edentata, and Ungulates, which anatomically are widely separated, as well as its absence in the placentiferous Didelphia are facts that should render us diffident in pronouncing the diffuse arrangement to be archaic, and that should encourage us to consider whether perhaps it might not be degenerative or secondary simplified, similarly as the omphaloidean placentation of the opossum is most probably a secondary simplification of arrangements such as we find them in *Perameles*.

In order to develop this more fully I will go back to Schoenfeld's latest contribution to the subject. In his comparative resumption of the facts established for the rabbit and the dog he says ('03, p. 813):

"In comparing the results obtained in these two mammals considerable analogies can be detected in the genesis of their placenta. I wish to throw full light on the absolutely passive part that is played by the epithelium of the uterus and by the uterine glands. Those elements are destroyed in the rabbit and the dog;¹ they give rise to *débris* which in the rabbit are resorbed by the plasmodium, but especially by maternal leucocytes and by decidual elements (glycogen cells), which in their turn degenerate and are resorbed by the foetal plasmodium; in the dog the *débris* of the glandular cells are resorbed either by the foetal plasmodium or by the trophoblast cells of the terminal plates.

¹ So they are, according to my own experience, in *Erinaceus*, *Tarsius*, *Tupaja*, *Sciurus*, *Sorex* (after a temporary proliferation of the maternal epithelium, which thus offers a more extensive pabulum to the destructive trophoblast cells; cf. Hubrecht, '94a), *Talpa*, *Galeopithecus*, *Vespertilio*, monkeys and man.

A second point which they have in common concerns the important part that is played in both cases by the foetal plasmodium. By its presence it brings about the destruction of the epithelium and of the glands; both in the rabbit and the dog it penetrates in the connectivo-vascular decidual tissue and reaches the maternal vessels, which it separates from their adventitial (decidual) cells.

If, however, in these two animals, the presence of the egg in the uterine cavity can somehow provoke a reaction on the part of the connective tissue that has become decidual, which is characterised by an active proliferation of its elements, then—at the same time there exists a considerable difference between the two species with respect to the evolution of the decidual cells. In the rabbit these last named are destroyed wherever they come in contact with the foetal tissue, particularly with the plasmodium: in the dog, on the contrary, the connective tissue-cells are not destroyed; they take part in the constitution of the plasmodium, they fuse with it, and give rise to a mixed plasmodium. The connective tissue-cells of the rabbit succumb in the struggle with the invading foetal elements, whereas in the dog they resist and associate themselves with the latter. The same is the case for the endothelium of the blood-vessels; it disappears in the rabbit, it persists in the dog. . . . The placental ectoblast (Hubrecht's trophoblast) is thus differentiated into a cytotrophoblast (inner) and a plasmoditrophoblast (outer layer). The plasmoditrophoblast adheres at a given moment against the uterine epithelial symplasma and brings about the disappearance, the destruction of the latter. The plasmoditrophoblast then penetrates into the modified uterine connective (decidual) tissue, which in Hubrecht's terminology is known as the trophospongia.

In invading the trophospongia the plasmoditrophoblast brings about the destruction of the latter in the rabbit, whereas in the dog it associates itself with it into a mixed plasmodium."

I have given this long citation because it is such a clear

resumption of the facts as they present themselves to those who are unwilling to fall in with Strahl's views, and because it allows us to group the other Insectivores and Rodents as far as known with the rabbit, whereas those carnivores, besides the dog, that have been carefully investigated up to now (cat, Putorius, fox) as well as most probably the bats (Fig. 140) all belong to the second category. Now if we remember the numerous points of comparison which the palæontologists have taught us to notice between early Carnivora (as have been the Creodonts) and early Ungulates (as were the Condylarths), then we are induced to consider whether in respect to placentation the arrangements which on this head are characteristic for the living representatives of both orders¹ also merge into each other.

A very strong argument in favour of the view here advocated is furnished by Assheton's researches ('06) on the placentation of Hyrax (vide Fig. 145.) Here we have a mammal that in many respects offers archaic peculiarities, and that has been placed not far from the Rodents (*Procaviidæ*!), from elephants, and from Ungulates by different

¹ Cretaceous tritubercular Creodonts are considered (vide Weber, 1904, p. 586) as having been parent forms, both of Condylarths and of other ungulate families beside these, and I presume that it was during this process of evolution that the early placental arrangement underwent the simplification which so naturally leads from the arrangements as we know them for living Carnivores to those so-called diffuse placental, but in reality aplacental, arrangements of the Ungulates in general.

Parallel phenomena of placental simplification occurred in two other great phyla of monodelphian mammals, most probably, however, at a yet much earlier period. This led up to the Lemurine so-called diffuse placenta on the one hand, to that of *Manis* (among Edentates) on the other. Cetacea, Proboscidea, Sirenia equally seem to be examples of a placentation, which, like that of the Carnivora (resp. early Creodonts), finds itself on the road towards simplification. I presume there is great probability that in their ancestral forms all these orders more closely resembled the present Insectivores and Primates, as far as the complication of their placenta went, but that their considerable increase in size favoured extension with simplification of the placental area, as this is more evident yet in Ungulates and Lemurs.

More details are given further on p. 144.

authors, and that—as far as its early placental characters go—resembles man, the anthropomorphæ, and the hedgehog, types whose placentation we were willing to regard as corresponding to primitive arrangements. The fact that modern palæontology (vide Weber, '04, p. 715) admits relationship between Hyrax and the fossil Condylarthra (Menicotheridæ), and even (“in einer entlegenen Wurzel”) with the South American fossil Toxodontia, brings the importance of the Hyrax placentation in general yet more to the front.

It should simultaneously be kept in view that the living Ungulates are ever so much further specialised from the Condylarthrs than are the living Carnivores from the Creodonts. This, of course, affords an *à priori* probability that the Carnivores have as yet less far departed from the original arrangement than have the Ungulates.

And with this *à priori* conclusion before our minds we will now consider the facts of the case.

d. Other Insectivores, Ungulates, Edentata, and Lemures.—In the Insectivores, also distantly related to Creodonts and Carnivores, but in many respects more primitive than the latter, we find a state of things in which the destructive faculties of the trophoblast come into play more fully yet than in the Carnivores, whereas in the Primates (Tarsius, monkeys, and man) that destructive faculty is present in quite unabated energy. If we look upon what Schoenfeld has above so well depicted for the dog as a modified, more benign process, originally derived from the first, but in which the endothelium of the maternal capillaries is spared by the destructive phagocytic trophoblast, while certain other maternal elements of the syncytium are also allowed to associate with the trophoblast without being destroyed, then we can imagine the same process yet further restricted. We would then have, for example, a denudation of the maternal mucosa, local or general, by the destruction of the maternal epithelium through the activity of the trophoblast, but the trophospongian reactions in the subepithelial maternal tissue might be reduced to a minimum, say to the

production of certain distinct cellular (decidual) elements, which might approach the denuded surface and eventually fuse with or pass through the adherent trophoblast cells. The maternal capillaria would then not either be eaten into, but also have preserved their endothelium, consequently the interaction between the maternal and the embryonic blood would be a little less direct, but this might be balanced by this somewhat less direct interaction taking place over a considerably extended surface, consequent upon the so much more considerable size of the blastocyst. Now if we consult the very recent paper by *Ciro Barbieri* ('06) on the placentation of *Tragulus meminna* we find a state of things there described which approaches closely to what was sketched above, viz. a denuded mucosa, an active trophoblast of which vascularised villi penetrate into denuded crypts, decidual maternal elements which pass from the mucosa into the trophoblast, thus forming an association of maternal and embryonic elements as in the dog, not, however, localised, but transitory.

The fact that the surface of the interchange between the *Tragulus* foetus and its mother has a more considerable surface extent than that in the dog and very much more than in any Insectivore should also not be lost sight of, especially when we consider that other species of *Tragulus* examined by *Selenka* ('91) and by *Strahl* ('05) show, again, further diminution of the destructive trophoblastic activity, because in these the maternal epithelium does not disappear in the crypts. The maternal and embryonic blood is in these cases separated by fully two epithelial strata, and the passage of decidual elements through the trophoblast was not noticed. There is, of course, not the least difficulty in passing from these latter stages on to those which we find both in Ruminants and in such Ungulates as the horse and the pig, which latter have always been looked upon as the prototype of the diffuse placenta.

Suppose this to have been the real phylogenetic development of the arrangement of the so-called "placenta" of Ungulates—which would thus in reality be a secondarily

simplified process in which the trophoblastic activity had considerably subsided—we would then have no difficulty in understanding that similar simplification and change of function, leading to parallel results, had occurred in other orders of mammals. As such we may count certain Edentata (*Manis*) and the Lemurs, although our actual acquaintance with the former is yet very scanty.

We know that in *Myrmecophaga* and *Dasypus* there is a discoid micrallantoidean placenta, that in *Orycteropus capensis* the placenta is zonary (as yet very imperfectly known), that in the sloths it has a more cotyledonary character, whereas in *Manis* more recent investigations (also extended to histological detail) of Max Weber ('91) have made us acquainted with a placentation very much like that of the simplified Ungulates, but at the same time with very marked vestiges of trophoblastic proliferation (Fig. 155). Considerable maternal proliferation of the uterine mucosa, such as was also figured for *Manis* by Weber, suggests the probability of descent with simplification from ancestors with more complicated arrangements. But the delicate question whether this latter proliferation is, indeed, maternal or—as has been proved in similar cases in other orders—trophoblastic will first have to be solved.

At all events, for the Edentata more extended researches on all the living genera should elucidate the question whether simplification in the direction of a so-called diffuse placenta is the real explanation of many of the facts here encountered. It should be borne in mind that any direct comparison of *Dasypus* on one hand and *Manis* on the other may be as misleading as that between Lemurs and Primates, because also on palæontological grounds the old order of Edentata is being split up into two or three independent ones, comprising, one, the *Nomarthra* (by Max Weber ['04] again sub-divided into the separate orders of *Pholidota* and *Tubulidentata*), the other the *Xenarthra*.

And now, in the third place, the Lemurs. Their so-called diffuse placenta, of which I gave figures fourteen years ago

('94 B, Figs. 31, 39, 40), is here represented by Figs. 146 and 152. It has since then also been studied by Strahl ('99), and offers different points by which it is differentiated from that of the Ungulates, as, for example the presence of capsular spaces (Fig. 152) which have been discussed by Strahl in his contribution to Hertwig's Handbuch. Chiromys (Fig. 151) has the same arrangement. We cannot for the present indicate the intermediate steps by which the simplification of a placenta of the Insectivorous or Primate type down to that of the present Lemurs was brought about and we may safely affirm that this secret has been taken into the grave by very old, probably mesozoic, Mammalia. But I hope that all the considerations we have discussed above may have sterilized any attempt to place Ungulates and Lemurs on one line, viz. that of the so-called primitive placentation. We are in no way justified to evolve the ever so much more intricate and perfected placental arrangements of Primates and Insectivores out of them.

3. Didelphia Placentalia.

We must now for a moment consider more closely the place which the placentiferous Didelphia have to occupy in this line of argumentation.

Without it being necessary to recapitulate the details furnished by comparative anatomy we may take it for granted that these mammals, which are now restricted to Australia and America (but in the tertiary period also spread over Europe), ought to be looked upon as an early side-branch of the mammalian stem, which has undergone very numerous adaptations to food and surroundings in its recent home, and which is characterised by peculiar points, both osteological and odontological, but more particularly by the curious physiological process of short pregnancy and very early birth that is followed by a protracted period of passive adhesiveness to the maternal nipple, generally inside a ventral brood-pouch.

Besides scanty details about their development which we

owe to Owen and others, our knowledge of their ontogeny has recently been furthered, in the first place, by Selenka ('87) and Hill ('97). And the researches of the latter, that have already been alluded to above more than once (p. 3), have shattered the old notion that this specialised group of mammals was intermediate between the Ornithodelphia and the Monodelphia. They have furnished most weighty data from which we must conclude that—previously to the very quaint modifications which have taken place when the growth of the foetus was in part transferred from the uterus to the marsupium—these animals were more closely related to monodelphian contemporaries than they are now. Most of them have now, during the short period of pregnancy, a well-developed area vasculosa on the umbilical vesicle, which, thanks to a quite extraordinary development of the proamnion (Fig. 130), can most efficiently serve as a means of osmotic exchange between the foetal blood and the maternal, which circulates in deep folds of the uterine mucosa. At the same time most of them show an allantois which lies hidden in a recess of the umbilical vesicle, and does not in any way come to the surface or partake in nutritory exchanges.

Hill's researches ('97, 1900) on *Perameles* and *Dasyurus*, and what Caldwell ('87) found many years ago in *Phascolarctos*, show that this passiveness of the allantois and its ineffective and hidden situation are not the general rule. In the genus *Perameles* the allantois partakes in a very effective placentation, histologically corresponding to what we observe in the Monodelphia; in *Phascolarctos* we notice a first step in a degenerative direction, the allantois yet touching in one circular spot the outer wall of the foetal vesicle, but not entering any more into vascularised connection with the mother.

We must now more fully discuss the earliest phenomena that are described by Hill for his *Perameles* blastocysts, more particularly as far as the proliferation of the trophoblast is concerned.

Hill comes to the conclusion ('97)—and Strahl ('06, p. 277)

has fully accepted this—that at the spot where in *Perameles* the allantois gives rise to the placental attachment the trophoblast—which in an early stage can be most sharply (Fig. 138) distinguished from the maternal trophospongian syncytium (into which the maternal epithelium has been converted)—disappears entirely in a later stage, presumably phagocytically destroyed by the maternal syncytium, thanks to which (Fig. 139) the maternal blood is now brought into very close contact with the embryonic blood circulating in the allantoidean vessels.

Now a phenomenon of this nature which, as even Strahl acknowledges, would be unique among the Mammalia, is far from being firmly established in Hill's paper. In many of his figures, which have not been copied by Strahl, and which may be said to represent transitory stages between his figs. 149 and 150, we see the trophoblast cells of fig. 149 becoming converted into much larger cellular elements (our Fig. 138), which, instead of being attacked and resorbed by the maternal syncytium, penetrate into this and very freely mix with it in a way which corresponds most closely with what Schoenfeld has so well described for the dog. I have no doubt that also *Perameles* offers a very good example of a syncytium with a double, mixed character, in which both maternal and foetal (trophoblastic) elements exist side by side of each other, and by which the endothelium of the maternal vessels is not attacked or eroded. Thus in the height of development of the *Perameles*' placenta (Fig. 139) we clearly recognise the presence of the trophoblastic elements yet in full activity, which at other spots are so mixed up with the maternal syncytial cells as to have given rise to the erroneous conclusion accepted by Strahl of the trophoblast's disappearance.¹ The name of semiplacenta avillosa by which Strahl designates it will have to be dropped. The *Perameles*' placenta may be said to be a somewhat simpler—because

¹ I have to thank Mr. Hill for sending me some of his original preparations of the placenta of *Perameles* in different stages, by which I have been enabled to confirm the contradictory opinion here formulated.

thinner—form of placenta than that of the Carnivora, but at the same time to approach most closely to that type, whereas amongst the Insectivora, *Sorex* provides us with an example (Hubrecht, '94A, Fig. 74) of a yet more extensive proliferation of the maternal uterine epithelium before the allantoidean attachment of the blastocyst comes about than even *Perameles*. At all events, the placentation of *Perameles*, characterised by so intimate a fusion between foetal and maternal elements, should never be classed amongst those forms of placenta which are either primarily primitive (as yet unknown to us) or secondarily simplified (Ungulates, Lemurs, Cetacea, etc.).

CHAPTER V.—DIFFERENT ASPECTS AND DETAILS OF PLACENTATION.

1. Embryonic (Trophoblastic) and Maternal (Trophospongian) Preparatory Processes.—We have in the preceding chapter followed the mammalian blastocyst in its very varied attempts to remain attached to the wall of the maternal mucosa; and we have seen that either part of or—in some exceptional cases—all the trophoblast-cells bring about this fixation by peculiar modifications. We once find the blastocyst attached either by its surface diametrically opposite the embryonic shield (*Tarsius*) or by the surface contiguous to the embryonic shield (*Lepus*, bats, mole, *Perameles* among *Didelphia*) or by both these surfaces together (catarrhine monkeys). Or, again, the blastocyst may be fixed in a zonary or ring-like shape, and the axis of this ring may be perpendicular to and below the embryonic shield (*Sorex*), or it may run parallel to the shield (Carnivores), or finally a double batch of proliferating trophoblast may be present, not, as in the catarrhine monkeys, above and below the developing embryo, but right and left of it (*Tupaja*). Again, the blastocyst may be fully enclosed in maternal tissue, and the trophoblastic activity may then

reveal itself all round (Erinaceus, man, and Anthrozoidea, many rodents), or the attachment of the blastocyst to the maternal mucosa may be so superficial that the trophoblastic proliferations (Fig. 134) serve other purposes than fixation (Opossum). Finally, in certain cases no trophoblastic proliferation is noticed at all (many Lemurs, Sus, Equus).

At all events, the trophoblastic fixation of the embryo is something essentially different from the fixation by means of a placenta, although in all cases the definite placenta becomes established at a spot where trophoblastic proliferation has paved the way, but by no means on all the spots where such proliferation has preceded. Some of these regions, as we have seen for the hedgehog, and as holds good for other mammals, serve the purpose in the form of the so-called omphaloidean placentation, others never come in direct contact with any vascular area of embryonic origin.

That part of the trophoblastic proliferation which corresponds with the spot where the definite placenta is going to be developed may be indicated on Duval's example as the ectoplacenta, so that in catarrhine Monkeys and Tupaja there is a double ectoplacenta, whereas in the hedgehog we might distinguish a ring-shaped omphaloidean and a disc- or saucer-shaped allantoidean ectoplacenta.

Placentation, properly speaking, only becomes a fact when this ectoplacenta which is vascularised by embryonic vessels and soaked by maternal blood, circulating in vessels or in lacunar spaces, has entered into such intimate fusion and concrescence with maternal trophospongian proliferation that the two tissues can no longer be distinguished, much less be separated from each other. In this way there is much to say in favour of denying the existence of such a thing as has been called "the diffuse placenta." Maternal and embryonic tissues being in that case so perfectly free and independent from each other, that at birth they separate as easily as a finger does out of a glove, a placenta cannot possibly be said to be present. Fusion of embryonic with maternal tissues is its *conditio sine qua non*, and so we must admit

a placenta in case of certain Didelphia (*Perameles*) and deny it to certain Monodelphia (*Equus*, *Sus*, *Nycticebus*, *Galago*, and others). Attempts at systematic arrangements based on placental characters having never been very successful up to now, there is no objection to this somewhat radical change in our conceptions.

And so in order to understand the final constitution of the placenta it is not sufficient to be acquainted with the very varied changes in the trophoblast which precede it, but it is also necessary to study most closely the diverse modifications and proliferations which take place in the maternal mucosa preparatory to the coalescence with distinct regions of the embryonic trophoblast. It would fall outside the scope of this paper to enter into a full and detailed description of all these varied modifications. I will only select a few examples in order to call attention to the extreme width of variation which this series of maternal preparatory arrangements for the confluence with the semi-parasitic trophoblastic tissues can undergo in different genera of mammals.

But before entering upon those details I wish to have formulated a generalisation to which a close comparison of all the facts observed in this whole field of inquiry necessarily leads us. Those facts then have established that the quintessence of the respective changes that become apparent in the maternal tissue consists in: (*a*) degeneration and destruction—sooner or later—of the uterine epithelium and of the uterine glands in the region of the future placenta; (*b*) increase of the vascular supply in that region; (*c*) production of tissues histologically resembling as closely as possible those which the trophoblast produces; fusion and concrecence being thus facilitated; (*d*) arrangements by which extravasation of blood in other directions than that of the trophoblastic lacunæ is rendered difficult or impossible; (*e*) in certain cases development of hæmatopoietic properties, the blood-corpuscles thus formed being set free in the maternal blood as are those produced by hæmatopoietic processes in certain trophoblast cells; (*f*) arrangements by

which, when once the regular passage of maternal blood into the trophoblast has been firmly and safely established, all these preparatory processes as far as the mother is concerned come to a standstill, the further elaboration of the placenta being exclusively a function of the trophoblast and of the embryonic blood-vessels or vascular allantoic villi, which gradually have become imbedded in and ensheathed by the trophoblast.

In short, we may say that the mutual relations between maternal trophospongia and embryonic trophoblast are such that the maternal trophospongia leads up to the formation of a hæmorrhage, and that the embryonic ectoplacenta (itself a trophoblastic proliferation), succeeds in surrounding this hæmorrhage most thoroughly and in utilising it most fruitfully. It was Duval ('89-'92) who first established this comparison.

a. Insectivora.—For the hedgehog we have in the preceding chapter given a full account of the phenomena accompanying the attachment of the blastocyst. We will here add a few facts concerning the maternal preparation for the placentary attachment.

Already on p. 102 the local swelling was noticed into the median pit-like cavity of which the early blastocyst disappears. These swellings arise after impregnation but independently of a local stimulus caused by the blastocyst, as I have more than one preparation in which the swelling is present but does not include a blastocyst. Another detail which proves the relative independence of these swellings is the very fixed and regular appearance of a limited hæmorrhage occurring at the lips of the swellings such as have been described by myself and by Resink, and by which the final closure and the completion of the decidua reflexa is brought about. Characteristic features of the trophospongean swellings here described are yet the following. They arise in the antimesometrical half of the mucosa and have the aspect of a spherical knob with an incisure on its free surface, the direction of which is parallel to the axis of the

uterus horn. A transverse section of this longitudinal incision, the lips of which coalesce when the decidua reflexa comes to be established, and shows the aspect given in Figs. 2, 3, 37 of my paper of '89. The cavity is thus not so much a cylindrical one (as it would seem to be if only one section is examined) but a slit-like one.¹

And the swelling itself is evidently one of interglandular, non-epithelial vascular tissue of the mucosa. Numerous fine capillaries transverse the swollen region in which the uterine glands and their lumen rapidly degenerate and disappear (cf. Hubrecht, '89, figs. 37 and 38), sometimes even (l. c. fig. 39) the remains of the glands being phagocytically disposed of by the activity of the trophoblast cells. The endothelium of these maternal capillaries is generally swollen; their opening up and the extrusion of the blood-fluid into the trophoblastic lacunæ after their having been eroded by the action of the trophoblastic cells has already been described above. The swelling continues to enlarge simultaneously with the enlarging blastocyst inside of it. The part of it which will contribute towards the constitution of the reflexa is the part which protrudes in the uterus lumen; it becomes thinner and its elements more stretched and fibrous as pregnancy goes on; finally it becomes, together with the trophoblast, a thin membrane, which ruptures at birth.

The remaining saucer-shaped portion of the maternal trophospongia takes part in both these successive phenomena of growth and of distension, but as it is applied against the antimesometrical wall of the mucosa it does not share the vicissitudes of the reflexa, but constitutes what has been called in human embryology the decidua serotina. It flattens

¹ It will be very interesting to learn whether in man the closure of the decidua capsularis comes about in the same way. It has as yet not been definitely established, although it seems very probable. N.B.—This footnote was already in print when, during the proof correction, I became acquainted with Bryce's and Teacher's sections of a very early human blastocyst ('08) which, even more than Peters' specimen, establishes the similarity which in this respect exists between man and the hedgehog, a similarity which I have ventured to predict in an earlier publication ('89).

out more and more, the trophoblast applied against it undergoing a series of modifications, fully described by me elsewhere ('89, Pl. 26), and thus forming the bulk of the placenta, in which allantoic villi form an intricate network supporting embryonic vessels. The blood contained in the latter is thus bathed by the maternal blood circulating ever since the beginning in the intervenient meshes of the trophoblastic meshwork.

The full-grown discoid placenta of the hedgehog is thus nearly exclusively a product of embryonic (trophoblastic) activity, and has gradually become evolved out of what was originally a thick spherical coating of trophoblast, closely comparable to what we notice in man (Fig. 143). When at birth it comes to be severed from the maternal mucosa and to be expelled as "afterbirth," a certain, though in no way considerable quantity of maternal tissue comes with it, the puerperium being accompanied by phenomena which have been more fully described by Strahl ('07).

After the hedgehog we will yet successively discuss of Insectivores, *Sorex* and *Tupaja*; of Chiroptera, *Vespertilio*; of Carnivores, the dog; of Rodents, *Lepus* and *Cavia*; of Primates, *Tarsius* and man.

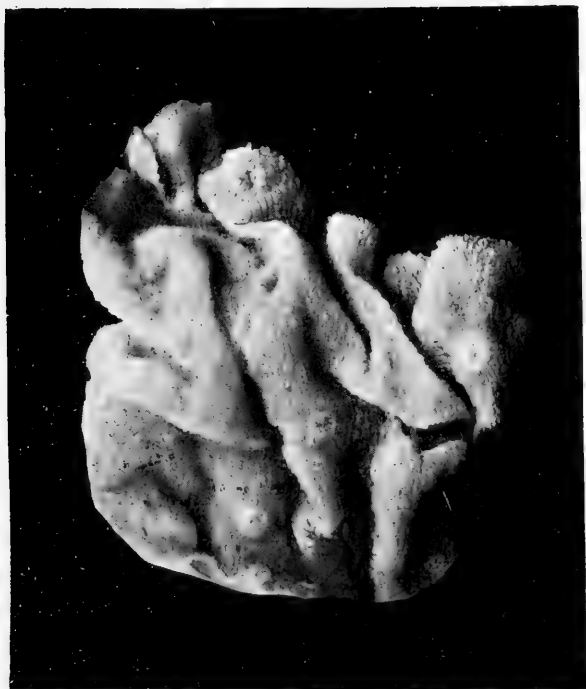
In *Sorex* the maternal trophospongian proliferation is exceptionally not in the first place subepithelial but epithelial. As I have described elsewhere ('94A), a considerable cushion of mucosal proliferation brings about the nearly cylindrical swelling against which (i. e., Figs. 8—11) the omphaloidean circulation of the embryo fits, whereas at the spot, diametrically opposite to the mesometrium, where the allantoidean placenta will later be situated, a very marked epithelial proliferation sets in. This proliferation soon becomes provided with crypts, which may on no account be confounded with the original glands, of which traces are co-existent with them. Into these crypts trophoblastic proliferations become ensheathed (Hubr., '94A, Figs. 74—81), and for a time maternal and embryonic proliferation are equally represented in this region until the embryonic becomes dominant,

when once the basis has been established for an intimate relation along a considerable surface between the maternal blood-corpuses circulating in the trophoblast and the embryonic ones also present in it. In this way, again, the placenta becomes established, the allantoic villi and their trophoblastic sheaths being spun out centripetally and not centrifugally. The massive dome-shaped placenta is thus in its full-grown condition, essentially again an embryonic structure in which maternal blood circulates (Hubr., '94A, Figs. 11—15); the maternal epithelial proliferation has gradually been reduced to flat remnants in the region where the maternal blood enters the trophoblastic lacunæ. Also in *Sorex* the placenta is expelled as afterbirth, and the regeneration of the mucosa comes about so quickly that young embryonic stages are often met with in a uterus which yet carries the unmistakable signs of the puerperium.¹

Tupaja is an example amongst Insectivores in which the disappearance of uterine glands in the region which will serve for the attachment of the placenta is not postponed till pregnancy has commenced and the formation of a maternal trophospongia has actually been inaugurated. Even in the virginal uterus of *Tupaja* that region can already be distinguished by the absence of glands. As *Tupaja* has a double placenta, right and left of the developing embryo, which is always situated with its head turned towards the ostium uteri and with its belly facing the mesometrical attachment of the uterus, and as moreover *Tupaja* never produces neither more nor less than two young at a time (Hubrecht, '95, p. 10) these predisposed spots are situated very symmetrically in the two uterine cornua. When pregnancy commences a general swelling of the uterine tissues is noted,

¹ One word may here be added concerning the mole's placenta (see Vernhout ['94] and Strahl ['90, '92]), which is not expelled as an after-birth, but which is resorbed in loco, embryonic trophoblastic tissue serving thus as a pabulum to maternal histolytic processes; the placenta, instead of deciduate, being thus, as I have termed it, contradeciduate, which term has been accepted by Hill ('98, p. 424) for *Perameles*.

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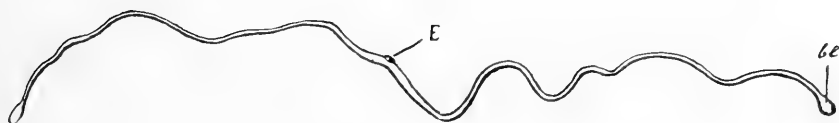


Fig. 153. External aspect of the villiferous blastocyst of the pig (after Strahl, '06). — Fig. 154. The elongated early blastocyst of the sheep (after Bonnet). — Fig. 155. The tropoblast of *Manis* with local proliferations (after Weber).

and the two spots here alluded to become very marked. They protrude with cushion-like convexity in the uterine lumen, and are covered by a pallisade epithelium, against which the blastocyst becomes attached. The trophoblast proliferates, as was noted above (Hubr., '99, Pls. 5 and 6), and as soon as the blastocyst has come to adhere to the two spots just mentioned the maternal epithelium is destroyed, and processes of mutual interlocking between the subepithelial maternal proliferation and the trophoblastical proliferation now ensue.

Here, again, as in *Erinaceus* and *Sorex*, the embryonic proliferation becomes very soon dominant when once the passage of maternal blood into trophoblastic spaces has been brought about by the aid of the maternal trophospongia, and now the allantoidean villi, ensheathed by trophoblast, continue by their further mutual growth to considerably thicken the incipient placenta. This thickening takes place here again in a centripetal direction. It should be remarked as a very wide difference between what is observed in *Tupaja* and what occurs in the hedgehog and the shrew, that the two pairs (one pair for each fœtus) of cushion-shaped spots of *Tupaja*, described above, first serve for an omphaloidean placentation, but that after a certain time the vascular area on the umbilical vesicle is dislodged out of its situation, its place being then taken by the allantois, which develops the villi ensheathed by trophoblast that were above alluded to.

The two placentas right and left are of course identical. They seem to be rarely expelled as afterbirth in toto, but rather to be broken up partially, perhaps even partly to be subject to a resorption in loco, as was noticed for the mole's placenta. These data I owe to Dr. Miss M. v. Herwerden ('06), who has lately looked through a series of preparations of puerperal *Tupaja* uteri.

b. Chiroptera, Carnivora, Rodentia.—In the Chiroptera the placentation has been studied by Frommel ('88), v. Beneden and Julin ('84), Göhre ('92), Nolf ('96), Duval ('99), and others. Here, too, there is a considerable amount of maternal trophospongian proliferation, which in many cases

invests as much as three-quarters of the surface (Fig. 159) of the blastocyst, but does not close up to a full decidua capsularis. The sequence and the histological detail of the phenomena are to a great extent comparable to what we saw in the hedgehog; for the details the authors above cited should be consulted.

For the Carnivora, Duval ('94, '95), Bonnet ('97, '01), Schoenfeld ('03) and others have furnished us with reliable data. Here, again, the definite placenta is a structure of embryonic derivation, which partly eats its way in the symplasmata resulting from the degeneration of the epithelium of the uterine glands. More so than in other orders of mammals certain maternal elements persist (see above, p. 108), though enclosed by the trophoblastic syncytium; it is even stated that the endothelium of the maternal capillaries is not destroyed, as is the case in so many other mammals. In this respect the arrangement in *Erinaceus* is more thorough.

Coming to the Rodents, Schoenfeld ('03), whose important researches have been alluded to above, has lately compared the rabbit with the dog, and comes to the conclusion that they have very much in common, the rabbit's placenta being, however, discoid, the dog's zonary. As to the histological differences, both show trophospongial (maternal) and trophoblastic (embryonic) preparatory processes before the blastocyst becomes attached to the uterine wall; after that the maternal epithelium is destroyed in the rabbit yet more fully than in the dog, also as concerns the endothelium of the maternal capillaries, which in the rabbit decidedly disappears under the destructive agency of the trophoblast-cells or their derivatives.

In the other Rodents we have already noticed the so-called Träger as a particular trophoblastic proliferation against which, after certain further cellular intermingling with maternal trophospongial elements the allantoidean placenta comes to be developed. The combined action of trophoblast and trophospongia brings about spacious lacunæ round the blastocyst in the earlier stages of pregnancy. In these lacunæ

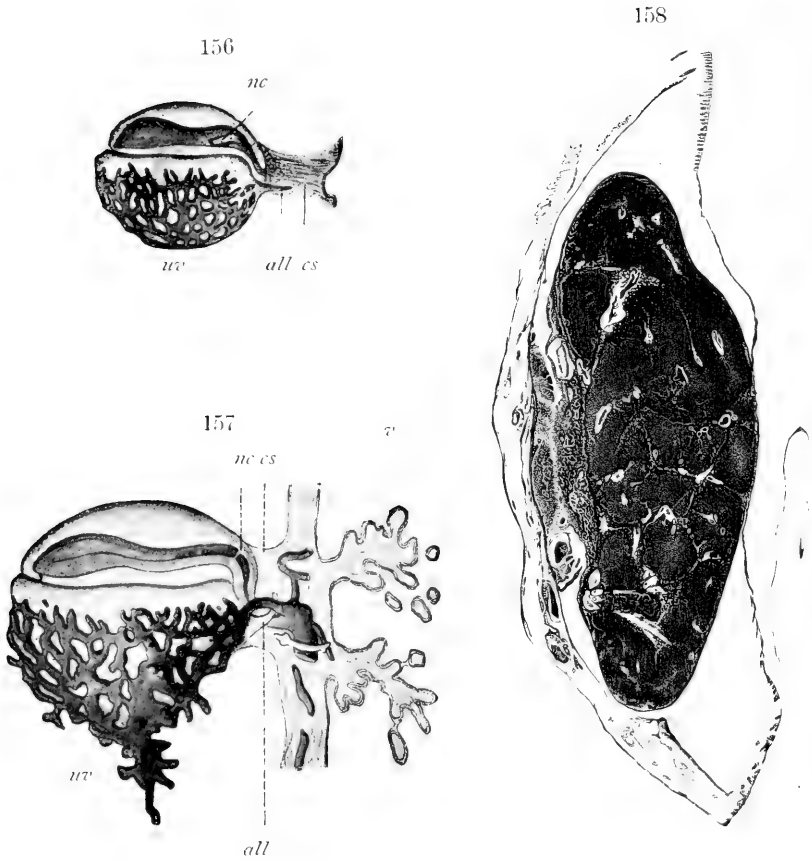


Fig. 156 and 157. Two stages in the development of the connective stalk and the umbilical vesicle of *Hylobates concolor* and *H. Rafflesii* (after Selenka, '00). The proliferating network on the umbilical vesicle is strongly developed and of haematopoietic significance. Vascularized trophoblastic villi are visible in Fig. 157. *cs* connective stalk, *v* placental trophoblastic villi, *uv* vascular network on the umbilical vesicle, *all* allantoic tube, *nc* neurenteric canal. — Fig. 158. Section through the stalked discoid placenta of *Cavia* (after Strahl, '06).

maternal blood circulates freely; later the nutritive processes are more concentrated in the placenta.

c. Primates.—Of Primates I will just yet touch upon the placenta of *Tarsius* and man. The first is the result of a limited trophoblastic proliferation simultaneous with a trophospongian process by which interglandular mucosal tissue prepares a surface with which the trophoblastic proliferation very soon forms a most intricate concrescence, in which maternal blood freely circulates, and which attains to comparatively considerable thickness before embryonic vessels have yet become ensheathed between the trophoblastic proliferations (Hb., '99, Pl. 11, fig. 67). Soon after this latter process begins, further increase only occurs in this trophoblast and its enclosed embryonic vessels, the trophospongia remaining active only in the zone where the placenta will separate from the maternal tissues, this zone being in the latter half of pregnancy only a stalk (Fig. 147) through which arteries and veins have access to the placental blood-spaces.

Such a stalked condition of the placenta is also characteristic (Fig. 158) for certain Rodents (mouse), and to a certain extent for *Sorex*, whereas in the squirrel, the hedgehog, in man, in *Galeopithecus*, and others the discoid placenta may be termed sessile over its whole proximal surface. Hæmatopoietic processes occurring in *Tarsius* during placentation have been noticed by me elsewhere ('99, p. 368, Pl. 14).

The placenta of man has already been alluded to on p. 101. There is no doubt that in it trophoblastic elements play quite an overwhelming part, much more so than was recognised by earlier observers (Figs. 142, 143). Thanks to the investigations of von Heukelom ('98), Peters ('99), and Bryce and Teacher ('08) we have now also become acquainted with part of the trophospongian arrangements in man, and a prediction of mine ('89) that the early, then as yet unknown stages of the human placentation, would offer close resemblance to what we find in the hedgehog has been fully confirmed by the authors alluded to.

One of the most notable differences between the placenta

of man and the hedgehog consists in the greater freedom and greater extension of those villi in which the embryonic blood circulates, bathed by the maternal blood in the trophoblastic lacunæ. These villi are in no way to be looked upon, as many of the text-books yet have it, as so many ingrowths which the chorion has sent out to penetrate into the maternal tissue. They are in man, and also in monkeys and *Tarsius*, growths not of a centrifugal but of a centripetal nature, as we have also had occasion to describe the corresponding structures in the hedgehog, in *Sorex*, *Tupaja*, etc. The freedom with which they float about in the maternal blood is another characteristic of man and the monkeys (Figs. 141, 142). In *Tarsius* and in the hedgehog their arrangement is more that of a suspension in a very delicate and at the same time most intricate trellis-work formed by the trophoblast cells that have become spun out into this. When the connecting trabeculæ of this trellis-work are suppressed, as we see it in the higher Primates, the surface available for osmotic interchange is naturally increased, and the free movements of the villi may also be considered as an advantageous circumstance (Fig. 144).

About the histological details of the placenta of man and monkeys certain points are yet in dispute, and such investigators as Selenka ('00A) and Strahl ('02, '04) seem to be willing to put to the account of maternal proliferation more than they are justified to. An agreement will, I expect, soon be reached, and the latest researches on these and other orders of mammals (Bryce, '08) seem to point in the direction, which Duval ('88) and myself ('88) have been indicating for the last twenty years, viz. final destruction of the maternal epithelium and circulation of the maternal blood in trophoblastic lacunæ.

The histological details of the placenta of the catarrhine monkeys resemble very closely those of man and the Anthropomorphæ. Whether their double placenta (Fig. 132) is a primitive or—as I hold it to be—a secondary arrangement (derived from an ancestral decidua capsularis) must be solved

by later comparative investigations of the more primitive Platyrrhines and Arctopithecii. Only lately Strahl ('06B) has recognised the presence of a decidua capsularis in *Mycetes*, a platyrrhine monkey!

2. The Classificatory Value of the Placenta.

The short account of diverse placentas in different orders of mammals in this and the preceding chapter can have convinced us of the inadequacy of judging about the more or less close agreement of these different placentas by their outward shape, as was done in the second half of the last century when the distinction of zonary, diffuse, and discoid placenta was first proposed, and when at the same time the now obsolete subdivision of the *Mammalia* placentalia into *Deciduata* and *Indeciduata* came into use.

The discoid placenta of the mole out of which at birth allantoic villi are retracted like so many fingers out of a glove and which is further resorbed *in situ*; the discoid placenta of *Galeopithecus* in which at the outset enormous lacunæ are filled with maternal blood and which at a later period is quite imbedded in the uterine wall; the discoid placenta of the rabbit and of *Tarsius* which, when full grown, is attached to the mother by a stalk of much smaller diameter than the placenta itself; the discoid placenta of the hedgehog and of man, the latter with its loose and floating villi as against the dense trellis-work of villi and trophoblast in the former; all of them are most intricate and very variously specialised, and at the same time essentially very temporary productions of these different mammals, the discoid shape being of no value whatever when considering their respective affinities.

To allow of the arrangement of the different types of placenta in anything like phylogenetic sequence the placentation of all living mammals should first be investigated and made known, and even then it will be very questionable whether the mutual relationship can be established to its full

extent now that the number of fossil mammals, about whose placentation we will never know anything, is so very much more considerable than that of the living representatives of the Mammiferi.¹ Especially the very early stages in the formation of the placenta and the mutual relation as well as the details of maternal trophospongia and embryonic trophoblast should guide us in comparing placentas and in deciding about their amount of similarity and homology.

And we will then certainly not be inclined to adopt Strahl's latest scheme for the arrangement of the different plans of structure of the placenta² ('06). The amount of blood-relationship which comparative anatomy (in its other chapters than that concerning placentation) enables us to establish between the different families of the mammalian stem obliges us to reject his plan of classification.

¹ The attempt lately made by Strahl ('06) to introduce a new classification, with a corresponding novel terminology for the mammalian placenta, is decidedly premature, and as such detrimental to real progress on this head. It condemns itself, where Strahl adduces ('06, p. 275) in its favour, "Dass wir nach derselben die bisher bekannten Placentarformen gut gegen einander abgrenzen können. Wir brauchen keine Uebergangsformen zu notieren . . ." and further, "Ausserdem schalte ich dabei vorläufig einige seltenere, mir aus eigener Anschauung nicht bekannte Placentarformen aus, wie sie gewissermaassen als Specialitäten in einzelnen Tieren vorkommen."

This immature attempt may appear satisfactory to its author—who in a later publication ('07, p. 19) has, however, already proposed certain corrections—but it breaks down (independently of the general considerations just brought forward) in the very primary subdivision into Half-placenta (Semi-placenta) and Full-placenta (Placenta), when we consider that, according to Strahl's own definition, the mole ought to be removed from the second, Perameles from the first subdivision.

The principles of Strahl's system are decidedly artificial, and may satisfy the anatomist who has to consider the human placenta in the light of comparative anatomy. But the zoologist, who considers only the phylogenetic development—so very difficult to construct—as a trustworthy guide to classification, will prefer to abide for the present, and to look forward for new data, before proposing a new classification for the so diverse phenomena of placentation.

² As, for example, where he classes together as *Mammalia choriata* *C. semiplacenta diffusa*: *Cetacea*, *Suidæ*, *Equidæ*, *Camelidæ*, *Manis*, *Tapir* *Hippopotamus*, *Lemures*.

3. The Phylogeny of the Placenta.

Although it may yet be too early to venture upon the attempt of sketching a phylogeny of the placenta, different from that in which the diffuse placenta is looked upon as the starting-point such as we find it generally accepted in text-books, still I may be allowed to bring forward certain considerations which ought to be kept sight of whenever that sketch is drawn up.

In the first place the old and catching comparison between the very early villiferous state of the human blastocyst, which in the phase of, for example, the so-called Reichert's ovum was said to pass ontogenetically through a diffuse phase to which the discoid stage only succeeded later, ought to be definitely got rid of, as I have already suggested long ago ('89, p. 339). The fact is that this so-called villiferous stage of the human ovum does not resemble the diffuse placenta at all because (1) Reichert's ovum is incomplete, and if complete would not have a villiferous but a sponge-like aspect, the so-called villi being actually transversely connected superficially (cf. Figs. 36—40); (2) in consequence of the presence of a decidua reflexa (capsularis) it is not freely suspended in the uterine cavity as are the blastocysts which show the so-called diffuse placentation; (3) there are no maternal crypts clothed with epithelium into which the villi fit, but these are directly bathed by maternal blood.

Once this comparison being discarded, we ought to look the question in the face whether the diffuse placentation as we find it in the horse, the pig, and the lemurs, does really represent the first step on the road that finally leads to the very complicate placental arrangement of man and other mammals. The three examples just named are in themselves sufficient to arouse a certain *à priori* suspicion. We could hardly expect that the most primitive type of placentation should be retained in an animal that is so eminently specialised as the horse. Nor in an order such as the Lemurs that

is by some looked upon as closely related to monkeys and man, but of which the placentation is so utterly different. And so we will have to look out for a probable cenogenetic explanation of these cases of so-called diffuse placentation, which were already discussed above (p. 113).

The first condition that should be fulfilled by a natural scheme of placental phylogeny is this, that the different families and orders of mammals should fit into it according to the degrees of relationship that have already been established by means of the other systems of their organisation. And in making an attempt in this direction it is natural that we should first ask what is the nature of the placentation in those mammals that may be looked upon as representing the more primitive types—the Didelphia, the Insectivores, the Rodentia, the Primates? We then find, as we have already in part discussed above, that the Didelphia furnish very conclusive evidence of their being very specialised descendants of the placental mammals, that even in those, in which there is no more any real placentation as in the Opossum, there is yet a very active proliferation of the trophoblast, and that in those which do retain placentation, or the traces of it, this placentation can be omphaloidean (*Dasyurus*) or allantoidean (*Perameles*). Finally that in this latter case intimate fusion on a phagocytic basis comes about between embryonic and maternal tissue.¹

If we examine the two other orders of more primitive Mammalia, that have been submitted to a more extensive inquiry as to their placentation, the Insectivores and the Rodents, we are immediately struck by a fact of prominent importance as compared to what we find in the so-called higher orders, the Carnivores, Ungulates, Chiroptera, etc., viz. a most considerable amount of diversity, both in the general outlines and in the details of placentation. This is well

¹ This holds good, whatever view we may be inclined to share: J. P. Hill's that the trophoblast is destroyed by the maternal syncytium, or my own that the trophoblast is the more active part, remnants of the maternal tissue being, however, persistent, as was also noted in many carnivores.

calculated to confirm us in our judgment that these orders are more primitive, and that in them the phenomenon of placentation has not yet come to be normalized to a particular type. Still, this conclusion is only of partial value, as we will by and by see that the diversity here alluded to is in one case characterised by high specialisation, in another by the appearance of peculiar characteristics, which throw light on certain general problems of placentation, whereas in others, again, types are represented which might furnish an argument to those who wish to subdivide the order of Insectivores in two or more independent orders.

At all events, we must conclude from the facts before us that the really simplest and earliest form of placentation is no more represented in any living genus of mammals, and we have to attempt to disentangle out of all the numerous data at our disposal the phylogenetic evolution which has gradually brought about the numerous forms now known to us.

When discussing the trophoblast on p. 18 of this treatise, we saw that a change of function, which must have occurred at a very early period, when this larval envelope contributed towards the retention of the blastocyst inside the genital ducts of the henceforth viviparous Protetrapod, in the first place developed adhesive qualities by which the blastocyst remained fixed to the uterine wall. We have supposed that a second parallel phenomenon was an increase in size of the larval trophoblast, precursory to the further development of the embryo proper. In consequence of this the adhesive surface would become of more considerable extent, and could be pressed more firmly against the maternal mucosa. If, at the same time, phagocytic properties become developed (which are now generally recognised to be characteristic for ever so many mammalian trophoblasts), then in addition the trophoblast layer might serve to hand over into the cavity enclosed within it material elaborated by it, which might in its turn serve towards the growth and nutrition of the embryonic cells (*s. str.*). For it is sufficiently known that both in the

glandular products contained in the lumen of the uterus, in its epithelium, in its subepithelial layers, and in its blood-vessels matter is available which can be easily transformed into such nutritive material for the embryo the moment a means of transport and elaboration of this material is available. That the trophoblast does serve as such is also recognised by all observers.

Now I hold it probable that the first and strongest claim to which the blastocyst had to answer, when viviparity gradually came about, was that of fixation. We will find a proof of that by and by when we come to discuss the phenomena in Lemures. The most natural arrangement for the attachment of a growing blastocyst that is passing outwards through a cylindrical uterus was the zonary attachment which would be the simplest possibility by which the two surfaces might adhere together. This has been retained in the Carnivora and some other mammals (*Elephas*, etc.), and as such seems to have a primary significance. When firm attachment could be combined with phagocytosis it would be a safer arrangement than phagocytical absorption of elements contained in the uterus lumen without firm attachment; in this latter case expulsion of the growing blastocyst might be a dangerous possibility. And so the firm zonary attachment, combined with destruction and digestion of the maternal uterine epithelium, might be the next step which we also find realised in the Carnivora, to which then and there is added further extension of the phagocytosis by the diverse processes which have been so carefully analysed and so lucidly described by Bonnet ('02). The maternal tissue—whether we accept Strahl's, Bonnet's, or Schoenfeld's views concerning the maternal epithelium and the trophoblast—is universally recognised to undergo catalytic changes, and to pass into a symplasma, towards the composition of which superficial epithelium, proliferated epithelium of crypts and glands, subepithelial connective tissue, leucocytes, and blood have all largely contributed. This symplasma, thus prepared, is thereby made fit for the phagocytic absorption by the

trophoblast cells, who again pass the food thus obtained either to the embryonic blood-corpuscles or into the cavities inside the trophoblast, be this umbilical sac or extra-embryonic cœlom.

The details of these physiological and complicated nutritory processes are still out of our grasp, and nevertheless they have undoubtedly very important significance by the side of more simple osmotic phenomena. Bonnet recognises ('02, p. 489) that the actual feeding of the trophoblast cells on albuminiferous symplasmata, on fat, and on the morphotic substances of the maternal blood, as it takes place under our eyes, considerably facilitates our understanding how the proteids which diffund with so much difficulty pass from the mother into the embryo. Similarly the supply of iron in the mammals which have no ferruginous yolk to fall back upon, and which nevertheless must take place in utero, becomes explicable in this manner.

I feel confident that these researches of Bonnet and others on the nutritive resources of the Carnivores are of the highest importance for a full understanding of the placentation process, of which the starting-point would then be the combination of adhesive and of phagocytic properties in the trophoblast cells. The same investigator, Bonnet, has in an anterior publication made us acquainted with the presence in the sheep's uterus of a substance which he has named "uterine milk."

It is in reality the product of catalytic processes of the same sort as those that were described above, and it differs from the material produced in the Carnivores only in this respect, that it has been set free into the uterus lumen. A transition stage between the two is, perhaps, that case of the *Tragulus* embryo (another *Ungulate* already cited on p. 113), in which formed elements were seen to pass out of the maternal connective tissue, through a layer of trophoblast into the embryonic tissues. At all events, there is an *à priori* probability that the arrangement in which organic detritus in the uterine lumen is being absorbed by the embryonic trophoblast is a later development from that in

which the primarily adhesive trophoblast began to combine phagocytosis with mere adhesiveness. As the total surface of the blastocyst increased, and as the adhesion became localised in the maternal carunculi and embryonic cotyledons, the remaining surface of the blastocyst developed such properties that the uterine milk was easily absorbed by its trophoblastic outer layer. Such a process seems to have been further again specialised in the pig and the Lemurs, in which certain sac-like receptacles (Figs. 152 and 153) serve for the reception of foodstuffs prepared by the maternal mucosa, and hoarded by the embryo in these pouches. But I continue to maintain that these were not primitive arrangements, but derived from those where, as in Carnivores, the foodstuffs were sought for yet inside the uterine mucosa (and not in the uterine lumen) by the proliferating phagocytic trophoblast.

Besides by the direct phagocytic process, nourishment and then especially oxygen is yet furnished to the embryonic blood-vessels by the osmotic processes which take place between the maternal blood and the embryonic; and we may perhaps say that there has been a certain amount of competition between the two systems as to which of them should be foremost in providing for the requirements of the internal parasite, the embryo. So differentiation and adaptation has run along very different lines, now specialising in one, now in the other of these two directions, but in some combining the effects of both. It is probable that in these latter the beneficial effect obtained was the maximum, and that this has at the same time revealed itself by higher development of the embryo in general. And if we try to class the mammals according to this principle I think we may arrive at making a very fair bid for a natural arrangement both as far as placental and other anatomical characters are concerned.

The early Carnivores have been united by palæontologists in the fossil order of Creodonts, relationships between these and the early Ungulates being recognised. Many recent Insectivores also reveal by different points their more primi-

tive character. And, as was hinted at above (pp. 108, 126), it is among Carnivores that we find, both as to fixation of the blastocyst and histological details of the placenta, what may be looked upon as yet undifferentiated arrangements. The phagocytic phenomena are in full swing. Osmotic exchanges between maternal and embryonic blood are possible on an extensive scale, both on the omphaloidean and on the allantoidean plan.

Now among Insectivores many placentas, which, as we know (see p. 132), are here so very varied, also come under this definition. The omphaloidean placentation of *Erinaceus* follows its course, and plays for some time an important part in bringing about osmotic exchanges. After some time it is stopped by removal of the area vasculosa that becomes folded up, and it is replaced by the allantoidean placenta. In the very earliest stages of the blastocyst phagocytosis has taken place on a most extensive scale and with undeniable intensity, eroding the maternal capillaria and digesting glandular and uterine epithelium in a manner which only finds its parallel among monkeys and man.

Still it remains an open question whether the hedgehog's placentation should be cited amongst the more primitive types. In the mole we find certain characteristics which in another direction seems to be primitive. Vernhout's investigation ('94) has brought to light a very extensive phagocytosis in the early stages of placentation. At the same time we notice in the mole what I have termed the contra-deciduate type (vide Hill, '97, p. 424) of placentation. In the mole the act of parturition has a very peculiar character by itself through the fact that the embryo is expelled out of the mother's womb only enveloped in the allantois with the fully extracted villi forming a woolly covering to that foetal involucrum. The trophoblast and all its proliferations, which have carried on such active phagocytosis, remains adherent to the uterine mucosa, and is neither wholly nor partially shed but gradually resorbed in situ by the mother's tissues, causing the external aspect of the uterus during the puerperium to have a similar

aspect as during pregnancy, only in the inverted sequence; the uteri with the smallest swelling being the furthest puerperial stages.

In this case the properties of fixation and phagocytosis characteristic for the mammalian trophoblast have been able to come into play on an extensive scale without occasioning any hæmorrhage in the mother, even leaving a certain pabulum behind of embryonic origin, the digestion of which may rather be of some advantage to the mother than the contrary. There is some reason to believe this arrangement in which there is no question yet of an afterbirth, but rather the contrary (hence the name of *contra-deciduata*) should be looked upon as a primitive arrangement. The more so as a similar phenomenon has been noticed by Hill in *Perameles* where the allantois, however, is not expelled together with the embryo as we saw was the case in the mole, but where in addition to the trophoblast the allantois also appears to be absorbed by the maternal tissue, thanks to the activity of migratory leucocytes described and figured by Hill. Having advocated ('95B, p. 118) the archaic significance of the arrangement in the mole, already before Hill found a similar phenomenon in a didelphian mammal, I must naturally emphasise my original contention after Hill's discovery in a mammalian order which, however much specialised it may have become, certainly contains representatives of an old stock. Since then the peculiar *contra-deciduate* characters have been noted for *Tupaja* (to a limited extent at least) by Dr. M. van Herwerden ('06).

In these early types we thus see that maternal phagocytosis in the placental regions keeps pace with embryonic phagocytosis. Nutrition by osmotic exchange has undergone a very marked reduction in the *Didelphia* as was discussed above (pp. 100, 115), the genera *Perameles*, *Phascolaretos*, and to some extent *Dasyurus* being perhaps yet the last in which the earlier arrangements have been preserved. In all the others the allantois has in a greater or lesser degree been reduced both in size and in amount of extension against the trophoblast.

The intra-uterine nutrition is no longer accompanied—as in the more primitive *Perameles*—by fixation of the blastocyst against the uterine wall, and there is only a very loose connection between vascular maternal folds of the mucosa and the vascularised surface of the umbilical vesicle. Moreover, this connection is only of a very short duration, parturition taking place after eight to fourteen days, and the peculiar specialised nutrition in the marsupium coming into play immediately after. Still the early blastocyst of the Opossum shows the spongy proliferation of the trophoblast (Fig. 134), of which we may certainly say that it can contribute towards the absorption and elaboration of fluid material contained in the uterine lumen. It does not reveal marked propensities towards direct phagocytical action. Selenka found its lacunæ ('87) filled with liquid which it most probably derived from the contents of the uterine glands that had found their way into the uterine lumen.

Summarising what we find in the *Didelphia* we may say: (1) in the more primitive forms: a well-fixed blastocyst which is united by a proliferating trophoblast to the syncytium that arose out of the maternal uterine epithelium. The blastocyst is nourished by the combined results of phagocytosis and of osmotic exchange between on the one hand an allantoic and an omphaloidean vascular network with, on the other hand, a maternal lacunar circulation in a syncytium of mixed derivation, the embryonic parts of which are resorbed by the maternal after parturition; (2) in the secondarily specialised forms: a blastocyst very loosely held between numerous and intricate maternal folds with which it enters into osmotic exchanges by means of an omphaloidean circulation on the faintly convex surface above the embryo without any villi corresponding to the maternal folds. Moreover, an early trophoblastic proliferation in which probably absorption of fluid material, taken from the uterine lumen, is of more importance than eventually additional phagocytotic phenomena.

In all existent genera of *Didelphia* the early ontogenetic

events and the different phases in the mutual relations of blastocyst and mucosa ought to be fully known in order to furnish us with all the data that can be brought to bear upon this important question. And it is to be fervently hoped that those genera that are very rapidly diminishing in number in their native land, some of them even on the verge of disappearance, may yet be fully investigated before they have been exterminated, and have thereby become as mute on this important point as are their fossil predecessors.

Turning back to the Monodelphia we notice that among the Insectivores another genus than the mole, above discussed, furnishes particular points of comparison with certain Didelphia. The genus I here allude to is *Sorex*, in which a localised strong proliferation of the uterine epithelium has been described by me ('94A, Figs. 74 and 80) into which allantoidean villi fit, which in that early stage very much resemble those that have been figured by Hill ('98, Pl. 33, figs. 28, 29) for *Perameles*. If the pregnancy of *Sorex* were to be brought to an early close in this very stage by a series of new adaptations as have occurred in the Didelphia the resemblance on general points between *Sorex* and *Perameles* would be certainly remarkable. The maternal epithelial proliferation of *Sorex*, however, does not give rise to a syncytium as in *Perameles*, but to a cellular agglomeration in which crypts appear, each of which harbours a trophoblastic villus with its core of vascularised allantoidean tissue.

The parallel cases which we have been able to institute between Didelphia on the one hand, certain Insectivores and Carnivores on the other hand, seem to encourage us when we pretend that a similar stage must have been the average degree of complication to which the earliest mammalian placentation corresponded, and that the so-called diffuse placentation which up to now has been looked upon as representing an early starting-point has wrongly usurped this place, as we will by-and-by demonstrate when we will advocate that the latter arrangement is an example of a

much specialised lateral offshoot in the line of development. To our picture of the eventual earliest arrangement we must yet add that the blastocyst itself must in that ancestral form have been characterised—on account of what we have so fully discussed in Chapter IV—by a very early local or total vascularisation of the trophoblast by means of a connective stalk which formed an *ab initio* connection between the embryonic shield and the trophoblast. No free allantois can have been present in the very earliest cases; this must have made its appearance only gradually, probably in consequence of the vascularisation of the connective stalk having been temporarily overtaken by the vascularisation in the anterior four-fifths of the annular zone of entoderm, where blood and vascular tissue was being formed out of the latter (cf. p. 34). The vascular area on the umbilical vesicle was thus brought at an early period in close contiguity with the vascular maternal mucosa and an early omphaloidean placentation may have been called forth out of what had primarily been a surface of hæmatopoietic significance in the ancestors.

At the same time the direct chorionic placentation came to be retarded. Later, however, overtaking the precocious omphaloidean placentation, again it supplanted the latter in the later phases of development. This gave rise to the first appearance of a free allantois.

That the partial vascularisation of the trophoblast by means of a primitive connective stalk is not merely a hypothetical possibility is proved by *Tarsius*, which corresponds to a transition stage as here imagined, its blastocyst being moreover situated in the uterine lumen.

The great advance which has been made by the other Primates (monkeys and man) is that in these the blastocyst becomes attached to the uterus by a more considerable surface, and that the resulting placenta—be it single or double—is not stalked as in *Tarsius* but sessile, whereas in the *Anthropomorphæ* and in man the very considerable difference from *Tarsius* is this, that the blastocyst quite disappears within the maternal tissue, and is by the formation of a

decidua reflexa quite removed out of the uterine lumen (Fig. 143). This phenomenon of encapsulisation inside the mucosa has appeared independently in more than one order of mammals, and can be observed in all its transition stages in different genera (*Vespertilio* [Fig. 159], Rodents, etc.).

The question may be raised—but cannot yet be solved for the present—whether perhaps the placentation of the catarrhine monkeys has not arisen by secondary modification out of one in which a distinct decidua reflexa existed. Different details seem to point in this direction; the investigation of the placentation of more genera of monkeys than have up to the present been subjected to research on this point is very desirable.

The removal of the developing blastocyst out of the uterine lumen and its total enclosure by a decidua capsularis is a phenomenon of all the more primary importance, as by it the phenomena of osmotic and of phagocytotic nutrition can be ever so much more intensified. It is clear that the removal out of the uterine lumen may mean a most profuse extravasation of blood all round the blastocyst, combined with constant renewal and circulation of this maternal blood, which is absolutely impossible as long as the blastocyst remains situated in the lumen of the uterus. Man and the man-apes, different genera of Rodents, as well as the hedgehog (*Erinaceus*) and *Gymnura* have realised this arrangement, of which later investigations may yet bring to light new examples. We are certainly justified to say that this phenomenon of the formation of a decidua capsularis must have made its first appearance already in a very early moment of the phylogeny of the placentary arrangements.

Diametrically opposed to the intensification of both phagocytotic and osmotic processes, as it is presented to us wherever a decidua capsularis has come to be developed, is another phenomenon which, by the very nature of it, excludes the combination of it with encapsulisation, viz. the early increase in size of the blastocyst, by which its total surface, in comparison to that of the actual embryonic surface, becomes ever

so much more extensive, and offers more copious opportunities for the absorption of nutritive material either out of the uterine lumen or, more indirectly, out of the vascularised mucosal surface, be this provided with an epithelium or deprived of it.

This state of things we find realised in Ungulata, in Cetacea, and in certain Edentates. Both for the sheep and the pig Bonnet and Keibel, and earlier authors before them, have made us acquainted with a most considerable growth in size of the sometimes even tubular blastocyst (Figs. 153 and 154), on the surface of which the embryonic shield only occupies a hardly visible space (total length of blastocyst 21 cm., breadth $1\frac{1}{2}$ mm.; length of corresponding embryonic shield 1 mm.). This considerable surface increase, which is also found in the Equidæ and other Ungulates which have hitherto been ranked as representatives of diffuse and polycotyledonary placentation, is thus seen to go parallel to a certain extent to a not inconsiderable increase in the size of the adult animal, with a corresponding increase in the size of the, generally bicornuate, uterus.

The conditions in which we find the free allantois in these Ungulates show that the considerable enlargement of the blastocyst has only commenced after a free allantois had already been evolved out of the earlier arrangements. Before the allantois has spread out against the inner surface of the diplotrophoblast, the outer trophoblastic investment has full occasion to be very active in elaborating and transporting the detritus in the uterine lumen, which has been termed "uterine milk," inside the cavity of the blastocyst. After the allantoic vascularisation of the diplotrophoblast has come about the latter becomes applied against the maternal surface, where at numerous, but independent, spots (so-called carunculæ), the tissue has been prepared by the formation of so-called cotyledons, into which fit groups of allantoic villi. In other Ungulates no cotyledons are present, but the maternal surface is thrown into a dense network of folds and crypts, into which corresponding folds or villi of the blasto-

cyst fit. In the case of the polycotyledonary placentation, osmotic and phagocytic absorption is yet combined, in that of the diffuse placentation of the horse it would seem as if the osmotic interchange between the maternal and embryonic blood (which takes place all over the extensive surface where the villi interlock in the crypts) has by far superseded phagocytic nutrition. There is an intact double epithelial layer, one maternal, one trophoblastic, that everywhere separates the two blood-fluids; nevertheless the considerable surface over which the two circulatory systems are in such very close proximity seems to make up for what is lost in exiguity of the separating membranes. And so the placental arrangements, as we find them in the horse, appear to me as an extreme state of specialisation of what in Carnivores, some Insectivores, and in *Didelphia* was a more primitive but a more complicated arrangement. The fixation of the blastocyst by means of adhesive and phagocytotic properties of the trophoblast cells seems to have been reduced to a minimum; the phagocytosis, which was certainly more active in the *Artiodactyla*, where also the fixation by means of the cotyledons was somewhat more firm, is in no way prominent in the horse, but the possibility of osmotic processes between large surfaces of maternal and foetal vascularised tissue has reached a higher degree of development.

The polycotyledonary arrangement has thus retained more hereditary points in common with the primitive placentation described above, than the diffuse. *Tragulus meminna* has already been cited (p. 113) in support of this. Also the less considerable degree of specialisation, which we find in the skeletal parts of the limbs, would correspond with the smaller amount of placental specialisation. The sequence in placental complication would thus have to be reversed; it is not the polycotyledonary arrangement that represents an advance as compared to the diffuse, but it is the diffuse that should be looked upon as the last rung of a ladder of simplification which the placental processes have undergone in the Ungulates, starting from the arrangements above alluded to, which,

though more complicated, were yet more archaic. The primitive earliest stages are unknown to us, and probably meant to remain unknown for ever, as so many transition forms that must have existed in the palæozoic epoch.

The "diffuse" placentation of the Lemurs should be looked upon as a second case of a simplified arrangement leading to a very similar result, as in the horse, but not necessarily, though not impossibly, along the same phylogenetic track. There is no reason why this simplification should not have arisen more than once; also in the Edentata, *Manis*, as has already been expressed above, gives another example of it.

That in Lemurs the evolution of the diffuse placenta has been different can be in part made probable by the fact of a very curious early phenomenon noticed in *Nycticebus*. We have already described in Insectivores, Rodents, and Carnivores the very early and very effective adhesion of the youngest blastocysts to the uterine wall, and the phenomena of placentation consequent upon this. *Nycticebus* has foetal investments which, in the latter half of the period of pregnancy, can, together with the enclosed foetus, be quite easily washed out of the maternal crypts, the trophoblastic villi not being in any way confluent with maternal tissue. There are two intact layers of epithelium between the maternal and the embryonic blood (Figs. 146 and 152). We would thus expect that the early blastocyst cannot either boast of any strong adhesion to the uterine wall, but would agree with the horse, pig (Fig. 153), sheep (Fig. 154), etc. *Nycticebus*, however, wholly differs from these latter by the fact that in those early stages, when the blastocyst has a diameter of 5—11 mm., it is very firmly kept in its place in the uterine horn, in which we find it, by another peculiarity. The horn (and the blastocyst inside of it) have, namely, undergone a quite unusual degree of distension; the median portion of the genital ducts, however, is not in any way comprehended in this enlargement. Consequently the blastocyst is kept in its place very effectually, although there is no surface adhesion whatever, and

although there are two intact epithelial surfaces in contact with each other, the uterine and the trophoblastic, which do not show as yet any wrinkling or any villi. Considering the presence of uterine glands, one might expect the surfaces to be lubricated by the secretion of these glands, and expulsion of the early blastocyst would undoubtedly follow had the swelling and extension not become limited to the horn only, in which, as I have described elsewhere ('07, p. 35), it is consequently generally very difficult to find the exact situation of the embryonic shield.

The difference in these early arrangements authorises us to keep the diffuse placentation of Lemurs apart from that of Ungulates. It was not necessarily obtained along the same hereditary line of development.

We have now sufficiently discussed the maximum degree of simplification which the placental phenomena undergo in Ungulates, Lemurs, and Edentates, to which attention had also already been called in the preceding chapter. In all of them an osmotic exchange between the contents of the capillary (not lacunar) circulation in the maternal mucosa and the foetal capillaries in the trophoblastic villi is obtained. The total surface over which this osmotic interchange takes place has become very considerable, and at the same time any concrescence between trophoblast and uterine epithelium has been quite given up, two intact epithelial layers separating the maternal from the embryonic blood.

We must now discuss some of the principal deviations from the central plan of placentation from which we started in opposite directions, viz. in such as bring about, instead of an extension of surface for the osmotic exchanges an intensification of the process over a restricted surface. This may, of course, be expected in those mammals which have not by an increase in the size of the adult (as in many Ungulates), so to say, created favourable conditions for surface extension in the placental processes. And, indeed, it is in Rodents, but especially in Insectivores and Primates, that we find intensified conditions as are here alluded to.

It has been noticed above (p. 103) that for such intensification of the osmotic exchanges the removal of the blastocyst out of the uterine lumen and its total inclusion within the mucosa by the formation of a so-called decidua reflexa or capsularis is very essential. The two most striking, and, at the same time, most perfect cases of this are presented (as was also already mentioned) by the hedgehog and by man. Still the two cases are in many respects different, but resemble in this respect that, whereas our primitive placental cases show a combination of phagocytosis and osmosis during a comparatively considerable portion of the period of pregnancy, in the hedgehog and in man the phagocytosis is of great intensity in the beginning, but is followed by a second period in which the osmotic interchange is considerably perfected. This latter perfection is noticeable along two lines. First, the tissue separating the maternal and the embryonic blood is most considerably reduced, and while we yet noticed two epithelial and two endothelial layers between maternal and embryonic blood in many Ungulates, we see that in Insectivores and Primates it may become reduced to a simple membrane of maximal tenuity. We need not insist upon the very great difference this makes for rendering osmotic interchange ever so much more effective, and we are then no doubt justified in saying that the Primates and certain Insectivores represent a step in advance on our archaic type, just as well as the Ungulates represented a retrograde step.

A second improvement by which intensification of the osmotic processes is being brought about, concerns the extent to which embryonic vascular surface is brought in contact with maternal blood. Here, too, we see that man and to a somewhat lesser extent the monkeys undoubtedly represent a maximum of intensification of the osmotic process. The allantoic villi, exceedingly numerous and finely branched and covered only by the excessively thin layer of tissue above alluded to, present an all the more considerable surface for the osmotic processes because they are freely suspended in the maternal

blood and thus bathed on all sides; whereas, for example, in *Tarsius*, in the hedgehog, and in other *Insectivores*, although there is only a very thin membrane separating maternal and foetal blood, still the section shows a very fine sponge-work of the finest membranous structures between which the allantoic villi are densely distributed. As they are, however, not freely suspended, but stretched between and supported by the meshwork here alluded to, the total surface available for osmotic interchange must necessarily be relatively less.

It would seem as if, in the human placenta, there is still left a certain margin for phagocytotic processes, brought about by the so-called "syncytial cells," which are present here and there on the villi, and are nothing but remnants of a plasmoditrophoblast (cf. Bryce and Teacher, '08). An important fact which was mentioned on p. 111 is the discovery by Assheton ('06) of the early placental stages in a primitive Ungulate as is *Hyrax*. It adds considerably to the probability that the simplification which was above suggested as having occurred in the phylogeny of the Ungulate placenta is, indeed, the actual explanation of the phenomena such as we notice them.

4. Summary of Chapters IV and V.

In concluding this and the preceding chapter I wish to emphasise that we have established an undeniable activity in the trophoblast of monodelphian and of didelphian mammals preceding and accompanying placentation, and that we have at the same time shown that those orders where such activity was insignificant or absent (*Lemurs*, certain *Edentates* and many *Ungulates*) must in this respect be looked upon as having been secondarily modified by various circumstances. Direct indications of this retrograde process are not wanting.

This being the case and the well-known and apparently natural starting-point which the so-called diffuse placenta offered us for establishing the phylogeny of placentation having thus broken down, we have attempted to establish

that phylogeny—about which palæontology will never be able to instruct us—on quite another basis.

This basis is far from being complete. Too little is yet known of the histological detail of the placentation process in the greater majority of mammals, and even when we will be fully acquainted with all those details as far as the recent mammals are concerned, even then we will perceive that the clue to many questions of phylogenetic importance lies amongst the extinct genera.

We may, however, say, that if on the one hand placentation details will help us to establish natural affinities in the grouping of the mammals, on the other hand no phylogeny of the placenta should be considered admissible if it would lead to any artificial grouping of naturally allied or naturally diverse mammals such as was discussed on p. 130.

Viviparity and placentation have gone hand in hand with the development of allantois and amnion. And only after the two latter had appeared in the early viviparous tetrapods of the palæozoic period did certain side lines of development diverge from that which led up to modern Mono- and Didelphia.

In these side lines oviparity again came to the front, and on them we meet the parent forms of the Ornithodelphia, the Reptilia, and the birds.

CHAPTER VI.—REFLEXIONS ON THE PHYLOGENY AND THE SYSTEMATIC ARRANGEMENT OF VERTEBRATES.

We have in the preceding chapters attempted to establish that certain fundamental conceptions concerning the embryonic envelopes and the placentation of the higher vertebrates are much in want of a renewed critical analysis. We have some time ago ('02, '05) come to a similar conclusion with respect to gastrulation in vertebrates.¹

¹ Keibel ('05) and Brachet ('05) have expressed their conviction that these

I will in this chapter attempt to draw the conclusions concerning the systematic arrangement of the vertebrates as such, to which due consideration of all the facts here considered must lead us, giving at the end a short sketch, partly already contained in earlier publications ('02, '05), of what may be considered as the most probable hypothetical invertebrate ancestors to which all these views point.

We have then first to take into account that the primary subdivision of the vertebrates is that into the two great groups sharply defined against each other as the Amniota (Mammalia, Sauropsida) and the Anamnia (Ichthyopsida). It has long been known that parallel and identical to this subdivision another is possible into Allantoidea and Analantoidea, and that the fact of the existence of this double character increased our faith in the significance of this primary subdivision of the vertebrates.

However, we have since seen that it would be difficult to pretend that the Primates are true Allantoidea, a free allantois not being present in this order. And on the other hand we have seen that of even more importance than either amnion or allantois is the outer embryonic layer, the trophoblast; in itself a larval envelope of very great antiquity.

The trophoblast, which is most marked in mammals, is ever so much more hidden in Sauropsida, and its presence can here only be recognised by a careful comparison of all the variations which we notice in its relations to the embryonic epiblast respectively in Mono-, Di-, and Ornithodelphia.

Clearer, however, than in most Sauropsida are certain reminiscences of the trophoblast in many Amphibia, Dipnoi, and Teleostomi. They consist in the presence, during early larval life, of an outer, generally somewhat more strongly pigmented, and also generally flattened layer of cells, which disappear when development proceeds, and which correspond, as far as

modified views seem to them to be more acceptable than the current opinions on the vertebrate gastrulation. This agreement is all the more welcome as Keibel, by his comprehensive article in the 'Ergebnisse der Anat. und Enter. gesch.,' vol. 10, has an authoritative voice in the matter.

their situation in relation to the rest of the embryo is concerned, with the trophoblast of mammals. In the Amphibia, Dipnoi, and Teleostomi, however, the layer does not in any way participate in the formation of an amnion or of a foetal envelope, nor does it remain at a distance from the developing embryo, protecting it in some way or other. Its significance as a transitory outer membrane is, however, undeniable, even when its participation in the formation of certain superficial, mostly larval, structures is remembered. And we are forced to consider whether we should not, for that reason, be justified in saying that, together with mammals and Sauropsids, these vertebrates have a common descent from ancestors in which a transitory larval envelope played a prominent part. We yet notice a similar occurrence in different classes of Vermes (Nemertea, Gephyrea) where certain groups have definite larval layers which are absent in others.

In that case a second consideration is this: do the cartilaginous fishes stand apart in that respect, and what about the Cyclostomes and *Amphioxus*?

About the absence in the latter genus of anything like an outer larval layer there can be no reasonable doubt after the numerous investigations concerning its early development which we owe to such a considerable number of trained embryologists. As to the sharks and rays, we can be equally positive that none of those who have studied their embryology up to now have cited any fact which would support the notion that anything like the "Deckschicht" of Teleostomes, Dipnoans, or Amphibia is present in any of them. We have, of course, the example of the Sauropsida to make us rather careful concerning cases in which there is an apparent absence of a trophoblastic layer. But then in this case the difference on many other points of comparative anatomy as between the cartilaginous fishes and the higher vertebrates is so considerable (as has already been partly pointed out above on p. 82) that it seems advisable to leave it open that the Selachians may very well have descended from ancestors without an outer larval layer.

For Cyclostomes the same reasoning holds good, although there are certain indications that in this group we have before us animals in which degeneration and regression with considerable modification has gone on to such an extent that it would perhaps not be impossible to link them on later to higher vertebrate ancestral forms as yet unknown.

And so the question presents itself:—Are we justified in displacing the dividing line which in vertebrate classification is almost generally adhered to¹ and which separates Ichthyopsida from Sauropsida and Mammalia? Or is it necessary to accept a primary division which brings together on one side the Cyclostomata and the Elasmobranchii, and on the other the Teleostomes, Dipnoi, Amphibians, Sauropsida, and Mammals?

I am well aware that I would not be justified to propose such a radical change only on the strength of the arguments which I have brought forward in this paper, and by which I have attempted to show that the second group is characterised by the more or less distinct presence of an additional larval layer, the trophoblast, whereas in the first group no traces of this have up to now been found.

But if we penetrate somewhat more deeply into the question by considering whether there are yet additional characteristics by which this dividing line might be strengthened, because also on other points the two groups are equally distinct from each other, then we may arrive at a firmer foundation in support of such a radical change.

In my opinion there are even two different lines of argument along which to advocate the new dividing line here proposed.

The first is offered by that series of organs which are so intimately connected with respiratory processes, and which we call the lungs and the air-bladder (swimming-bladder). After Spengel's ('04) and Goette's ('04) lucid articles there

¹ I must make an exception for Ray Lankester's article on Vertebrata in the 'Encyclopædia Britannica,' in which, with prophetic insight, he entirely ignores this subdivision.

can hardly be any more doubt but that we may look upon all the diverse modifications of lungs and swimming-bladder (the latter either double, ventral, or single and dorsal) as derivatives of what were originally a pair of posterior gill-pouches, in which change of function was slowly inaugurated parallel to preparatory steps by which an adaptation to terrestrial life was rendered possible.

Now the structures here alluded to are found in the Teleostomes, the Dipnoi, the Amphibia, Sauropsida, and Mammals, and never was any trace of them found in the Elasmobranchs or the Cyclostomes; so that here we have a concomitant argument to the one derived from the trophoblast in further justifying the new line of demarcation.

And I would call the attention of those who hesitate to introduce this new barrier between cartilaginous and osseous fishes to a set of other considerations which in my opinion have not been sufficiently looked into up to now.

It is this, that while nobody objects to the Cetacea being looked upon as the descendants of terrestrial Mammalia, nor to the Sauropterygia and Ichthyopterygia as having sprung from Reptilia that were air-breathing land-animals, the question has not enough been looked in the face whether many of our Dipnoi, Ganoids, and Teleosts may not also perhaps have had terrestrial ancestors? I fully recognise that we are here entering a field of wild and hypothetical speculation, but on the other hand insist on the necessity of testing this heuristic assumption. If we admit that air-breathing, hairy, and milk-producing quadrupeds originally lived on the dry land and have been able secondarily to adapt themselves in the most marvellous way to a life absolutely bound in all its functions to the high seas as that of the whales, how could we then wonder that in the palæozoic epoch, when for the first time life on the dry land became possible and weird amphibious protetrapods left the water and managed to adapt themselves to this atmospheric environment, on many an occasion side branches of these earliest land-animals turned back to purely aquatic life

carrying certain hereditary stigmata which pointed to the fact that once they had been air-breathers already.

Up to now we only know such a ridiculously small portion of all the fossil animals that have lived in the palæozoic period, that it is not foolhardy to predict that very numerous remains may yet in future be unearthed in which this question presents itself.

And if we think of those innumerable series of species, genera, families, and orders of which at present we know nothing, is it then improbable that in those earlier periods of the world's history the same phenomenon of a secondary return to the aquatic medium has presented itself over and over again?

If I were allowed to point to one example I would select *Polypterus*, and ask if its paired and ventral air-bladder might perhaps not have served as effectual lungs to a more fully air-breathing ancestor, and if Klaatsch's hypothesis ('96) of the phylogeny of its limb-skeleton might not easily be turned the other way round so that the central plate with the two longer bones right and left of it should not be looked upon with Klaatsch as an incipient carpus with lateral radius and ulna, but as an adaptation of what had already functioned as a supporting limb-pair in a terrestrial ancestor to a re-assumed aquatic life?

Similar questions might be put concerning the *Dipnoi*, who in the Devonian epoch appear to have had—judging from footprints—five-toed tetrapod contemporaries. Even in *Teleosts* (*Saccobranchus* and *Anabas scandens*) evolutionary processes are going on even now which tend to an exchange of the aquatic for the atmospheric life and vice versa.

The air-bladder in the *Teleosts*—which by common consent is now generally derived from arrangements such as they are now possessed e. g. by *Polypterus*, and not vice versa—has this other curious particularity that in certain closely allied species of the genera *Scomber*, *Sebastes*, *Umbrina*, *Thynnus*, *Chironectes*, it may be totally absent in the one, present in the other. Thus according to Stannius, '*Zootomie der Fische*,' 2e

Aufl., 1854, S. 22, *Scomberesox Camperi* has an air-bladder, *Scomberesox Rondeletii* has none. In other families, *Squamipennes*, *Tænioidei*, *Siluroidei*, *Cyprinoides*, *Clupeidæ*, etc., the same is noted. I hold this to be an argument for looking upon the air-bladder as an organ that is fairly on the way to become rudimentary. Certainly not as an organ that is yet very essential to the life of many Teleost fishes in their present environment.

At the same time the fact of the existence of such a very great number of Teleost species is certainly no argument that the whole of their pedigree must necessarily lie in the aquatic medium.¹

I will not go so far as to say that all Teleostomes and Dipnoi have descended from terrestrial, air-breathing tetrapods, because the material upon which to base a similar conclusion is by far too scanty; but on the other hand I will not either for the same reason anathematise any naturalist who feels inclined to go as far as that. It should certainly be kept in view that the incipient aeropneustic conditions which ensued upon the adaptation of posterior gill-clefts to aerial respiration need not necessarily have been accompanied by a terrestrial life. Still it will certainly have contributed to render further adaptations to a terrestrial or rather amphibious existence easier.

I must, however, yet allude to one argument which goes parallel to that derived from the air-bladder and lung-arrangement.

It is an osteological argument and calls our attention to the fact that the mutual relation of the ossifications on the skull and visceral arches of the Teleostomes are to such a

¹ While correcting the proof of these pages Assheton's 'Development of *Gymnarchus niloticus*' (the Budgett Memorial volume, 1908) comes into my hands, in which I find the possibility of similar inverse relations discussed on arguments derived not only from lung and air-bladder, but on further developmental details concerning the vascular system and the gills, brought together under ten heads (l. c., p. 407). *Gymnarchus* belonging to a primitive family of *Malacopterygii*, it is only natural that I should welcome support obtained independently along a perfectly different chain of reasoning.

very great extent homologous both in number, in sequence, in position, and in development to similar ossifications in the Amphibia, the Sauropsids, and the Mammalia.

Confining ourselves to the comparative osteology of the head we may say that the conformity is very suggestive, and that, where nobody advocates any direct descent of the land-animals from Teleosts, this conformity might certainly plead for the possibility of the inverse proposition.

This proposition to be taken in the sense above alluded to, viz. that great attention should be given to the evident probability that the return to an aquatic environment may have been by polyphyletic lines of descent and at different periods of the earth's history.

There is no doubt that we must look towards palæontology for furnishing us with the arguments that will have decisive weight in deciding these delicate questions of phylogeny, for which we can never hope to possess arguments derived from splanchnological or from developmental sources.

And we may at all events expect that as more and new fossil finds come to increase our knowledge of the palæozoic epoch, some of them will certainly prove to have a bearing on the points here in dispute.

A division of the vertebrates in the superclasses of Cyclostomata, Chondrophora, and Osteophora might suggest itself, Amphioxus remaining yet more isolated in its superclass of Cephalochordata.

The Chondrophora would then contain the Elasmobranchs, the Osteophora all the other higher vertebrates.

In further subdividing the Osteophora the existent grouping into Teleostomi, Dipnoi, Amphibia, Sauropsida and Mammalia might remain, although it will have to be carefully considered whether the recent, most considerable progress of palæontology will not allow of a more satisfactory reclassification in the borderland between Amphibia and Reptilia, now that we have reason to believe that the very sharp distinction which in later days was upheld between these two according to the

presence or absence of amnion and allantois is to a great extent artificial.

When embryology no longer forces us to go on extending the distinction between the so-called Amniota and Anamnia into the palæozoic period, certain lines in comparative anatomy may perhaps suggest a new grouping in which also that other inadequate test, the double or single occipital condyle, is relegated to its real value. But then the palæontologist who will go deeper into this matter should bear two other points in mind which both this investigation and numerous other researches in comparative anatomy have brought to light of late years, viz. that the mammalian characteristics bring us down to a point where comparison with the lower Amphibia—as Fürbringer ('00) has more especially advocated—is more ad rem than comparison with the more specialised reptiles; secondly that the Ornithodelphia should be looked upon as a sub-class by itself, small at present, but perhaps more extensive long ago (Multituberculata), in which sauropsidan and mammalian characters are curiously combined but which was never in the direct line of descent of Mono- and Didelphia.¹ Then, again, that these latter may be said to be a very specialised side branch of ancestors that were

¹ I wish here to refer to a passage in an interesting article by Wortman ('03) on the origin of mammals (l. c., p. 429). He says, "Early in the mesozoic there appeared small, mammal-like forms, which were widely distributed over both the northern and southern hemispheres. Representatives of these species continued throughout the Cretaceous, and finally disappeared in the early stages of the tertiary. . . . Many of them are classified in the group Multituberculata, which, without much doubt, finds its nearest living representative in the Duckbill of Australia. . . . In one instance a fairly complete skull is known (Tritylodon) from the Karoo-beds of South Africa. The teeth of this species are astonishingly like those of many types in the northern hemisphere, and hitherto it has always been classified in this group. Seeley has shown that the organisation of the skull presents so many reptilian characters as to cause him to refer it to the Reptilia. If this reference is correct, then, in the absence of any fact to the contrary, it is highly probable that all the multituberculates are as much reptile as mammal. Indeed, it is not easy to say, at first glance, upon which side of the line living monotremes should be placed. There can be little doubt that, when more

already placental Monodelphia, so that the Mammalia s. str. are no more broken up into three stems but in reality contain only one, the course of which through the corridors of time will have to be established by the palæontologists, who will undoubtedly finally be able to trace it far into the carboniferous, nay, perhaps, even into earlier geological epochs, simultaneously with the first evolution of air-breathing vertebrates of Protetrapodian structure.

Then, again, if we take these Monodelphia, their respective subdivision into natural orders will awaken all the more interest as they bring us closer to the phylogenetic development of man himself, one of the problems about which the human mind will never be wholly at rest; and here comparative anatomy, embryology and palæontology ought to co-operate more intensely than it has hitherto generally done. Only of late—thanks, in the first place, to efforts of American palæontologists—this is brought home to us and is beginning to be realised.

Here, too, however, a very broad and very modern spirit ought to prevail. And though recognising that only of the recent Mammalia the embryology can be traced, and that there is not the least chance of ever obtaining positive facts concerning the embryology of fossil groups, still, it ought to be fully realised that when once the ontogeny of all the existing genera of mammals is known—and this is a goal that ought to be taken in view without delay—we will have in those facts indications of great delicacy for determining degrees of consanguinity. The details of ontogeny and

fully known, these ancient fossil types will present every conceivable gradation between these two great divisions of the Vertebrata."

Now, this is the very point which, on repeated occasions ('95, '02), I have advocated, viz. that the recent Ornithodelphia are one of the many offshoots into which the Protetrapodan ancestors have subdivided themselves, when once they had commenced to adapt themselves to life on dry land and to aerial respiration. The stems that remained viviparous are yet represented by the living Mammalia, those that have become oviparous diverged into the Ornithodelphia, and—further off yet—into numerous Reptilia, and have never given rise to viviparous descendants.

placentation will prove to be a very subtle instrument (as it has already shown itself to be with respect to the Primates) by which wide deviations in external habitat may be spanned and by which important generalisations may thus be reached.¹

Already have the voices of anatomists of different countries repeated what I have ventured to express more than ten years ago, viz. that among mammals the Primates have actually retained many very primitive characters. And the voices alluded to go even further and say that among the Primates the same may be said, in very many respects, concerning man as compared to the other Primates. Always with this one all-important reserve that his specialisation (*a*) in respect to brain development (including cerebral circulation) and brain power, (*b*) to adaptation of the forelimbs to the most diverse uses, and (*c*) of the larynx and tongue to articulate speech is quite out of comparison as regards importance with any other series of specialisations that are, however, so numerous amongst the different orders of mammals.

The order of the Insectivora will have to be broken up, and many of the small fossil mammals that may yet be brought to light will have to be carefully tested as to their relations to the different orders into which the Insectivora will be subdivided. Already Wortman has proposed to transfer the Hyopsodidæ (hitherto considered as Primates) to the Insectivora.

¹ I may here once more repeat, what I have already stated elsewhere, that placentation is so delicate a touchstone, because it was a phenomenon that appeared and evolved ever so much later than the other processes or structures in the vertebrate organisation, and that this comparative youth must decidedly contribute to retain small differences, which in older organs have been worn away by the effect of time. On the other hand, the details of the very early blastocyst must undoubtedly be of pre-eminent importance, just because they come to light at such a very early stage of development. The different characteristic details of very early stages must be all-important for determining hereditary affinities one way or the other, as they are undoubtedly least of all affected by influences that call forth adaptations in the organs of the adult animals.

And *Tarsius* will have to be definitely removed from the Lemurs, as has also already been done by myself ('96, '99, '02) and by Wortman ('03, '04, p. 167), who unites it with monkeys and man in the order of the Anthropoidea, differentiated from the Lemurs, in addition to the characters derived from the blastocyst and the placenta, discussed above, by the arrangement of the ento-carotid circulation, which in the Lemurs more closely approaches to the peculiar plan of the Insectivores.

Wortman's subdivision of his suborder of Anthropoidea in the three superfamilies

- (a) The Arctopithecini, including as single family the Hapalidæ;
- (b) Palæopithecini, including, besides *Anaptomorphus* and *Tarsius*, yet *Necrolemur* and (perhaps) *Microchærus*;
- (c) The Neopithecini, man and the living monkeys, besides the fossil family of Adapidæ,

is the embodiment of what I have stood up for since my publication in Gegenbaur's *Festschrift* ('96), and has, of course, my full sympathy.

I must, however, differ from Wortman when he considers "the Primates a perfectly natural and homogeneous order, including the Lemurs, monkeys and apes, as well as man himself" (l. c., p. 163). I hold his suborder of Anthropoidea, above named and very fully discussed in his paper ('03), to be in reality a full-rank order, which should retain the time-honoured name of Primates. The two other suborders which Wortman combines with his Anthropoidea, viz. the Lemuridea and the Chiromyidea, should be ranked together as suborders of the distinct order of Lemurs. I will discuss this point somewhat more fully with reference to the contents of the previous chapters.

Chiromys madagascariensis has a typical diffuse placenta, of which I here give a figure (Fig. 151) taken by myself from a *Chiromys* fœtus in the British Museum kindly lent to me for the purpose by the trustees. This placenta, which, as dis-

cussed on p. 115, can hardly be called a placenta at all, corresponds with the villiferous diplotrophoblast, with massive villi of *Nycticebus* in all respects, and I have no doubt but that also the relation between diplotrophoblast and allantois, etc., in *Chiromys* will be of the same type as that of *Nycticebus* (Fig. 148), so that really nothing is in the way of following Wortman's suggestion and placing these two suborders of *Chiromyoidea* and *Lemuroidea* together, selecting for the order which comprises them the name of *Lemures* as above stated. Besides the recent *Chiromys madagascariensis*, Wortman adds the fossil genera *Mixodectes*, *Cynodontomys*, *Microsyops*, *Smilodectes*, and *Metachiromys*, in all of which the dentition has acquired that peculiar Rodent-like aspect which is so characteristic for the recent genus. I prefer Wortman's views to the proposal which Osborn has made, viz. to unite the six American fossil genera into a suborder of the *Rodentia*, which Osborn calls the *Proglires*. Wortman states that what is known of the skeleton betrays the same *Primate* stamp with equal distinctness, as does the skeleton of *Chiromys*. And as to the modification of the incisors which is complete in the living Madagascar species, it is progressive but incomplete in the American genera. Wortman adds that "these are the only representatives of the *Primates* in which the slightest tendency towards such modification is shown. That so distinctive and profound a change could have originated twice independently in the same order is so highly improbable as to be unworthy of serious consideration." The group is of prætertiary origin, *Mixodectes*, its oldest representative being already highly modified in the second stage of the lower Eocene.

The *Lemuroidea*, which may be united with the *Chiromyoidea* into the order of *Lemures* are characterised by Wortman in the following manner :

"Limbs elongate, prehensile, and adapted to an arboreal habit; incisors of lower jaw reduced in size, pectinate and proclivous in position; anterior lower premolar very generally enlarged and functioning as a canine; ento-carotid canal

not traversing the petrotympanic; molar and lachrymal very generally in contact on anterior rim of orbit; fourth digit of the manus the longest of the series."

He adds: "Some . . . are inclined to deny the genetic connection of this group, as well as that of the Chiromyoidea with the true monkeys, and assign to them a separate and independent ordinal rank. This, however, is manifestly incorrect." After a page devoted to internal anatomy and placentation, Wortman concludes: "It seems by far the safest plan to rely largely if not solely upon osteological evidence for our conclusions respecting the affinities and evolution of the various groups of the Mammalia." And then he finishes by brushing aside the objections I have raised to connecting Lemures and Primates in one and the same order.

In once more vindicating the position which I have taken up in this question twelve years ago ('96) I may begin with remarking that the last citation, however comprehensible the idea there developed may seem from a purely palæontological point of view, is not justified in this particular case. Rarely have differences of such importance concerning internal anatomy been established as is the case between the two sub-orders of the Lemurs on the one hand—Tarsius, monkeys, and man on the other.

Wortman seems to have grasped these differences only partially, and writes: "It is difficult to decide what value is to be attached to the placentation in estimating affinities." In the preceding chapters we have repeatedly shown—as had been already done by me before Wortman published his paper—that it is certainly not only on arguments drawn from the placentation that Lemurs and Primates ought to be separated, although the placentation as such is, indeed, profoundly different in the two cases. But the difference between the evolution of the blastocyst, the part played by endoderm and mesoderm in coating the inner surface of the trophoblast and the way in which the diplotrophoblast is vascularised in a greater or lesser degree in the Primates, with the permanent retention of what we have called the

connective stalk, is so utterly different from what we find in Lemurs, and on the other hand so closely homologous if we take forms so wide apart as *Tarsius* and man, that we must frankly recognise that if ever, then here is a case in which these details of internal anatomy, as revealed by ontogeny, must weigh very heavily in the scale.

Wortman has not taken the least notice of the very important differences in the early blastocyst, and takes it easy with the placental differences as we have seen by the citation on p. 162. He even commits himself to the following statement (l. c., p. 403): "While it is probably true that these characters derived from the soft anatomy indicate a wide distinction between existing monkeys and lemurs, yet it is much to be doubted whether these distinctions would not assume very small proportions or completely disappear, did we have an Eocene monkey with which to make the comparison." Now this piece of reasoning is very lame indeed. We have an Eocene monkey to compare with *Tarsius*, viz. *Anaptomorphus*. On p. 213 of another publication ('04) Wortman (who places both in the same suborder as monkeys and man) enumerates eleven points of resemblance between *Tarsius* and *Anaptomorphus* (1) in size, (2) in brain development, (3) in relation of brain to foramen magnum, (4) in absence of sagittal crest, (5) in shortend face and large orbits, (6) in situation of internal carotid canal, (7) in dentition, (8) in structure of molars and premolars, (9) in shape of bulla, (10) in lachrymal bone and lachrymal opening, (11) in relations of lachrymal and malar.

Now, where these numerous points of resemblance exist it would be most illogical to presume, without strong positive evidence, that blastocyst and placenta of the Eocene *Anaptomorphus* as compared to the living *Tarsius*, were as wide apart as is that of a true Lemur like *Nycticebus* from that of *Tarsius*, as Wortman would have us believe. Moreover, this would not be an advance in any respect, because we do not see our way to derive the arrangements in *Tarsius* from those present in *Nycticebus*.

And so both the facts and the reasoning, that is brought to bear upon them, convince us that there is an immense amount of probability, that already in the Eocene did those fundamental ontogenic differences exist between the Primates as represented by *Anaptomorphus* and between the then existing Lemurs, which we now notice between *Tarsius* and the modern Lemurs, Ungulates, etc.

I hope to have established in the preceding chapters my full right at exacting the application of all data we dispose of, both osteological and ontogenetical, to the settlement of questions of affinity between Mammalia. That in very many cases, when groups that are exclusively fossil come under consideration, we will have to go by the osteological characters only, is, of course, self-evident. But it does not diminish our conviction that if there, too, we could have had ontogenetical evidence in addition to go by, our conclusions would be yet more emphatically trustworthy.

In the case of the Primates it is all the more necessary to insist upon the ontogenetical characters being allowed to have their full weight for several reasons. Firstly, because a careful consideration of these characters makes it evident that man, the monkeys, and *Tarsius* are more primitive in the possession of their connective stalk than are the Lemuroids with their free allantois, whatever may up to now have been said of the latter's placentation being more primitive, a point which in Chapter V I have endeavoured to reduce to its true proportions. Secondly, because the osteological characters seem to be such as to induce most palæontologists to incline towards a perfectly gradual passage from the lemuroid to the anthropoidean type. The facts of ontogeny, however, should force them henceforth to look out for additional characters by which Wortman's *Anthropoidea* (already represented in the Eocene by *Anaptomorphus*, for which there is no reason at all to suppose that its blastocyst was not as similar to that of *Tarsius* as its dental and skeletal characters are) can yet additionally be distinguished in older formations from those forms which must have led up to the

present lemurs. That Wortman unites the Adapidæ to the Primates s. str. and gives them not a subordinal rank but classes them as a family of equal value as the Cebidæ, the Cercopithecidæ, the Simidæ, and the Hominidæ is an important step, the justification of which can be better appreciated by trained palæontologists than by myself. But if Wortman is right in thus separating the Adapidæ from the Lemuridæ, Nesopithecidæ, and Megaladapidæ, which are the super-families in which he subdivides his Lemuridæ, then he and others will have to trace downwards the line by which, on the one hand, these latter families and, on the other, the Primates (Adapidæ included as above stated) are connected to earlier mammals of the Mesozoic in which the deep cleft which ontogeny demonstrates between the two may have been less, but traces of which must be deciphered out of osteological details.¹ Perhaps that problem may prove to be too arduous, but even then we are in no way justified to follow Wortman when he proclaims his sole faith in osteological characters and voluntarily suppresses ontogenetic evidence where it exists, because in so many cases it does not exist or rather can never any more be brought to add its testimony to what osteology reveals us.

I must in conclusion yet refer to a citation which Wortman gives from Flower and Lydekker's "Mammals, Living and Extinct." Wortman is quite justified in thereby (l. c. '03,

¹ Nesopithecus of Forsyth Major is an instructive example in this respect. Dr. Forsyth Major, from the unusually high development of the skull, and its many resemblances to the higher apes, concluded that it was an Anthropoid. Lydekker preferred to class it as a highly developed Lemuroid. Wortman followed him in this, undoubtedly after careful consideration of both Major's and Lydekker's argumentation, and instituted the super-family of Nesopithecidæ above referred to. Now, I have no doubt that the ontogenetical details of Nesopithecus would immediately have settled this question. As it is, it seems to me that only a most careful examination of the entire skeleton, wherever available, will furnish material for a definite judgment.

In the meantime we should, in this and other cases of so delicate and yet so important a nature, suspend our judgment, however much I would in the present case be willing to accept the validity of Lydekker's opinion.

p. 403) giving his reader the impression that the value of the deciduate and non-deciduate type of placenta has been overrated. Not only that, but since then among the so-called deciduate placental mammals some have been detected (Hubrecht, Hill) in which the placenta instead of being deciduate might even be termed contra-deciduate, in this sense that no maternal tissue is being expelled after parturition, but that embryonic tissue is undergoing a process of resorption on the part of the mother.

And so I will not deny that the value which we ascribe to particular points in the placentation and in the puerperium of mammals may vary according to the greater or lesser acquaintance we possess of their details. But I cannot overlook that even Flower and Lydekker in the same citation maintain that "the characters and arrangements of the foetal structures . . . will form, especially when more completely understood, valuable aids in the study of the natural affinities and evolution of the mammalia."

On p. 159 I have developed the idea that in certain cases the "character and the arrangement of the foetal structures" will even prove to be a discriminating re-agent both delicate and powerful. And I must emphatically repeat that the case of the ordinal separation of the Lemurs from the Primates is one of crucial importance, and that, whatever inconvenience may in the present state of our knowledge be caused by it to palæontologists, we should on no account surrender or acquiesce to the proposal of so eminent an authority as Wortman, but should determine (1) to keep separated the two orders of the Primates and the Lemures, and (2) to use all our ingenuity and acuteness in order to trace, as new fossil remains come to light, remains belonging to the one and to the other order by osteological details only. But then of course such finds which bring us teeth or even teeth and skulls only, may in some cases be misleading, and only complete skeletons can have full demonstrative weight.

This new and more exacting method of dealing with fossil remains is in the nature of things in the first place applicable

to Primates and Lemures, because we have concluded from the facts discussed in the preceding chapters that, more even than Huxley ('81) supposed the Insectivores to be, the Primates come under our consideration as containing the more primitive types of mammals. And it is only natural that they and their nearest allies will be more difficult to differentiate from each other than other mammals, who, even though archaic in some respects, were well specialised in others, as are many of the earlier Condylarths, Ungulates, and Creodonts.

Wortman, in his remarkable discussion on the origin of the Primates ('03, pp. 419—436) shares this opinion when he says: "It is true that the Insectivora furnish a type of cerebral circulation which might easily have passed into that of the Anthropoidea, through the suppression and disappearance of the stapedia branch of the ento-carotid, but, as we have already seen, this character is shared by the Rodentia, and probably by other groups as well. At the same time it does not form a type of cerebral circulation from which that of the Lemurs could have been evolved (l. c., p. 436)."

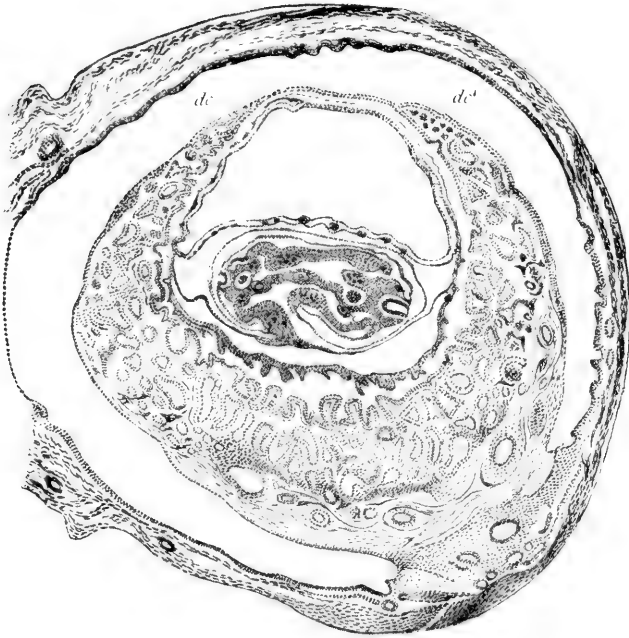
We here have a most instructive example of that differential treatment of the most delicate marks visible on the base of the skull of fossil mammals by which their ordinal grouping may be influenced. And it is exactly this delicate discrimination which I have been advocating in the preceding pages. The example is all the more instructive as it shows us points of agreement in angiological and osteological detail between Insectivora, Rodentia, and Primates, s. str., between which orders (all of them primitive) we have discussed so many points of comparison referring to the placenta, the blastocyst, etc. At the same time Wortman denies a similar degree of direct comparability on this particular point between Insectivores and Lemurs (see also l. c., p. 167), which, as he says, "are sufficiently distinct to afford reliable diagnostic characters." Now we have seen that also with respect to their peculiar placentation (which, as I have said, is not necessarily as primitive as it has always been looked upon)

the Lemurs differ considerably from Rodents, Insectivores, and Primates, but have again great similarity with Perissodactyles and Artiodactyles (*Equus*, *Sus*, *Tapirus*) and others. Here, then, is a point to which palæontologists should try to give particular attention. They might then help us to get hold of a clue by which to differentiate the early mesozoic pedigree of Ungulates and Lemurs from that of the Primates, Insectivores, and Rodents, and by that contribute to restore their own belief in the value of ontogenetical characters as guides to problems of classification.

I finally wish to cite the last phrases of Wortman's so exceedingly suggestive paper just alluded to, in which he finds it difficult—and I am here in full accordance with him—to derive the Primates from the Insectivora. He says: "The greatest difficulty in the way of deriving the Primates from any form or forms of the Insectivora at present known consists in the total lack of prehensile powers of the manus or pes. Any group which is placed ancestral to the Primates must of necessity be one in which some distinct approach to this condition is made, since its possession is one of the chief requisites of fundamental importance. . . . With the single exception of *Lophiomys* among the Rodentia, the only other living mammals which exhibit prehensile extremities are found among the Marsupials, and the evidence points very conclusively to the fact that all of them, even those with highly modified limbs for terrestrial progression (as the kangaroos), are descended from ancestors with grasping hands and feet. It is, therefore, not beyond legitimate supposition to assume the existence of a very considerable group of ancient Metatherians living within the arctic circle during cretaceous time whose manner of life had already become arboreal. If such a group did exist it is far more likely that the Primates were derived from it rather than from the Insectivora or any other group now living."

Now the prehensile power of the manus or pes lacking in the Insectivora as far as known to us re-appears again, at least in the shape of an opposable thumb in certain Amphibia

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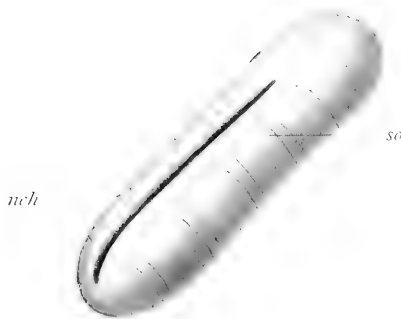


Fig. 159. Section through uterus and early embryo of *Pteropus* (after Göhre). There is no entire decidua capsularis. The placenta will be of the stalked type. *dc dc'* the free borders, which, if united, would form a closed decidua capsularis. — Fig. 160. Diagrammatic representation of a vermactinian stage in the phylogeny of vertebrates; the notochord *nch* developing out of the stomodaeum, the coelomic diverticula *so* some becoming split off from the enteron (after Hubrecht, '05).

and even the fossil amphibian, known only by its footprints, *Chirotherium*, is supposed to have possessed this distinctive character of the Primates. So here, again, we find an indication that in trying to bridge the distance between Primates and the lowest Tetrapods the road is rather *viâ* an amphibian ancestor than *viâ* reptilian-insectivorean transitional stages.

We now come to the final paragraph of this chapter, in which we have to give our attention to the continuation of the Vertebrate (Chordate) pedigree among the invertebrate phyla.

I have on a former occasion ('05A) expressed myself on this subject, but will here once more restate my own views.

The diagrammatic type for a representative of the vertebrate phylum would be a bilaterally symmetrical, segmented, coelomate animal, with the gut below, the central nervous system above the axial notochord. Following Sedgwick in his proposal to derive this type from an elongated, actinian-like starting-point, I have constructed a diagrammatic figure, which I here reproduce (Fig. 160), and in which I suppose the circumoral nerve-ring of the actinian-like ancestor to have become the central nervous system, the stomodæum to have become the notochord, the coelom to have arisen out of the peripheral parts of the coelenteron. The original actinian-like dorsal mouth-slit (itself a differentiation of what was the blastopore of the gastrula-larva) is only evanescently reproduced during vertebrate development by the communication between outer world and enteron, which travels backwards and separates the two halves of the concrescent notochord. The anus and the anterior neuropore may be two remnants of this slit; the vertebrate mouth is a neoformation, as are the gill-slits and the coelomopores. In how far openings in the lateral body-wall, by which in certain living actinians the coelenteron communicates with the exterior, belong to the same category as the openings just mentioned cannot for the present be definitely settled; nor can we know how many coelomic pouches were present at the starting, nor how

metamerism has finally increased after the gastrula stage had been passed and the phenomena of kephalogenesis and of notogenesis had begun to show themselves.

For an eventual comparison of the larval coelomic pouches as they were described for *Balanoglossus* by Bateson ('86) with what we know about the coelomogenesis in Vertebrates the indications at the present moment are only of the very slightest, too slight for making any further mention of them here.

The presence of an outer larval layer (of ectodermal derivation) in the worm-like transition-form that stood between this archaic starting-point and the predecessors of our osteophorous vertebrates, its absence in that which led up to the Cephalochordata, the Cyclostomes, and the Chondrophora was discussed on p. 151 of this paper.

A comparison of these hypothetical and intermediate stages between the coelenterate and the vertebrate phyla with the conclusions to which Woltereck has come, when he, too ('04), has stated that in Annelid development phases occur which seem to agree with what I have designated by the terms of kephalo- and notogenesis, had better be put off to a later publication, this last paragraph being more of a recapitulative than of a constructive significance.

I finally call attention to the fact that the unsatisfactory state in which our modern comparative embryology leaves a number of important phylogenetic problems—I may here call attention to O. Hertwig's own words on p. 898 of vol. I. 1. 1. of his new handbook—may partly find its explanation in the circumstance that up to now the comparative embryology of Vertebrates has been principally founded on what we knew of the chick, supplemented by what Kowalevsky and Hatschek taught us about *Amphioxus*, Balfour about Elasmobranch fishes. Now that we have proposed to accentuate the distinction between Chondrophora and Osteophora I may be allowed to invite younger embryologists to tackle wherever they can the early developmental stages of mammals or

Amphibia in preference to the cartilaginous fishes or to Amphioxus, however much more easy the latter material can be obtained.

I have no doubt that in mammalian embryology very many surprises are yet in store for us.

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Some Observations on the Infusoria Parasitic in Cephalopoda.

By

C. Clifford Dobell,

Fellow of Trinity College, Cambridge; Balfour Student in the University.

With Plate 1.

INTRODUCTION.

4 The infusorian parasites of cuttlefish are already well known from the excellent descriptions of their discoverer, Fœttinger (5, 6), and the admirable figures of Gonder (7). From the work of the latter it would appear that the last word had been said about their morphology. But, as they are of particular interest on account of the peculiarities of their nuclear apparatus, I took the opportunity afforded by a recent stay in Naples (March to June, 1908) of re-examining these organisms. The results were somewhat unexpected, and are embodied in the following pages.

OCCURRENCE OF THE PARASITES.

As is well known, three different Infusoria occur in cephalopods—*Opalinopsis sepiolæ*, *Chromidina* (*Benedenia*) *elegans*, and *C. (B.) coronata*.¹ The first—*O. sepiolæ*—has been recorded from the liver of *Sepiola rondeletii* (Fœttinger, Gonder) and from the liver of *Octopus tetracirrhus* (Fœttinger). Although I have examined fifty-five individuals of *Sepiola rondeletii*, I have never once met with the parasite. But I have encountered it in a hitherto unrecorded host,—*Sepia officinalis*,—and not only in the liver, but also in the kidneys.

¹ Following Gonder's nomenclature. *O. octopi*, Fœtt., is, as Gonder says, almost certainly identical with *O. sepiolæ*. Bütschli (2) united *Opalinopsis*, Fœtt., and *Benedenia*, Fœtt. (= *Chromidina*, Gonder) into one genus—*Opalinopsis*.

C. elegans was found by Føettinger and Gonder in the kidneys of *Sepia elegans*, and by Gonder in the kidneys of *Illex coindetii* also. I have met with it in both these hosts—though rarely in *S. elegans*. I have also found it in *Sepia orbignyana*.

C. coronata was found by Føettinger in the kidneys of *Octopus vulgaris*, and by Gonder in *Eledone aldrovandi*. I have found it only in *Illex coindetii*. I examined seven other species of cuttlefish in addition to those already mentioned, but with negative results. The results of the examination of all the cuttlefish is shown in the accompanying table.

TABLE.

CEPHALOPOD.	Number of individuals examined.	Number infected with		
		<i>C. elegans.</i>	<i>C. coronata.</i>	<i>O. sepiolæ.</i>
1. <i>Sepia officinalis</i> , L. .	73	0	0	1
2. <i>Sepia orbignyana</i> , Fér. .	8	1	0	0
3. <i>Sepia elegans</i> , d'Orb. .	82	4	0	0
4. <i>Sepiola rondeletii</i> (Gesn.), Leach.	55	0	0	0
5. <i>Illex coindetii</i> , Ver. .	9	5	2	0
6. <i>Octopus di filippi</i> , Ver. .	7	0	0	0
7. <i>Octopus vulgaris</i> , Lam. .	8	0	0	0
8. <i>Octopus macropus</i> , Risso .	2	0	0	0
9. <i>Loligo vulgaris</i> , Lam. .	13	0	0	0
10. <i>Loligo marmoræ</i> , Ver. .	16	0	0	0
11. <i>Eledone moschata</i> , Lam. .	24	0	0	0
12. <i>Eledone aldrovandi</i> , Raf. .	2	0	0	0
13. <i>Ocythoe tuberculata</i> , Fér.	3	0	0	0
14. <i>Rossia macrosoma</i> (D. Ch.), d'Orb.	7	0	0	0

The great scarcity of the parasites is remarkable. Out of 309 cephalopods examined only eleven were infected—i. e. about 3·5 per cent.

It is possible that the organisms occurring in different hosts are different species, but it seems to me unlikely. Assuming that there are but three species of Infusoria, their occurrence may be briefly summed up as follows :

Parasites.	Hosts.
1. <i>Opalinopsis sepiolæ</i> .	<i>Sepiola rondeletii</i> (liver). <i>Octopus tetracirrhus</i> (liver). <i>Sepia officinalis</i> (liver and kidneys).
2. <i>Chromidina elegans</i> .	<i>Sepia elegans</i> (kidneys). <i>Illex coindetii</i> (kidneys). <i>Sepia orbignyana</i> (kidneys).
3. <i>Chromidina coronata</i> .	<i>Octopus vulgaris</i> (kidneys). <i>Eledone aldrovandi</i> (kidneys). <i>Illex coindetii</i> (kidneys).

This table combines all the results of the work of Fœttinger, Gonder, and myself as regards hosts. It may be noted that all the work on these organisms has been done upon material obtained from the Gulf of Naples.

Chromidina elegans, Fœtt. emend. Gonder.

The general morphology of this infusorian has been accurately described by Fœttinger and Gonder. I will here record only those points in which my observations are in disagreement with those of these two investigators.

A point which does not seem to have been noticed previously is that the body is not uniform in shape throughout its whole length. Immediately behind the head there is a

very well-marked flattening, so that in this region a transverse section would be elliptical—not circular. This feature is so distinctly seen in the living animal, and so characteristic, that it is really surprising that it should have escaped notice. (Cf. fig. 3.) The animal swims with great rapidity, and invariably with the head in advance.

Gonder was the first to find that a cytostome is sometimes present in *Chromidina*. "For the most part one finds these *Infusoria* . . . without any trace at all of a cytostome. Only by more exact observation does one notice, in a small number, a cleft at the extremity, or at another spot on the anterior end." He "also found *Chromidinæ* with a completely developed cytostome—though these were, of course, less common." (7, pp. 246—7.) Now I believe that this statement results from the circumstance that Gonder was dealing largely with young forms of *Chromidina*. The "rudimentary cytostomes" are truly rudimentary in the individual, though not in the species. For I have found that every large animal possesses a cytostome—and a well developed one. As is well known, a *Chromidina* reproduces by constricting off small portions of itself at the posterior end, which then become free, and develop into new individuals. Usually these portions are described as "buds"; but they are more correctly termed segments—being formed, not by budding, but by a process of segmentation, like the proglottids of a tape worm. It follows that a young *Chromidina*, just freed from its parent, begins life without a mouth. Hence we find all stages in the development of this organella if we examine individuals at different periods of growth. (Cf. figs. 4, 5, 6.) The constant presence of a mouth in the organism is of importance for understanding the nuclear apparatus.

The vacuoles of *Chromidina* are non-contractile.

Nuclear Apparatus.—This is the most important feature, regarding which I differ from the other observers. I will first briefly summarise what has already been said about it.

According to Fœttinger, there exists "at times but a single nucleus . . . When there are several nuclear bodies these are merely fragments of the single nucleus. The latter, being capable of amœboid movements, may assume the most varied forms—push out extensions, become segmented, etc." To this account Gonder added that the nuclear substance undergoes a series of vegetative changes, so far as he was able to follow them, like those of *Opalinopsis* (see *infra*). He believes, further, "that those stages which we find in the posterior part of the cell or in the buds are the younger, those which take place in the anterior part of the cell the older." A cycle of nuclear changes is thus to be observed in one and the same animal at one and the same time. At first there is an arrangement of the chromatin in irregular fragments of different sizes. These then become converted into a network, which may then undergo a resolution into a system of strands, and finally give rise to a condition in which we see, once more, a number of irregular fragments. The net may also, it would seem, give rise to coarsely granular chromatin masses. Still another stage is described, but its relation to the others is not quite clear. It is a stage in which the chromatin and plastin re-arrange themselves in the form of a number of perfect nuclei—each with its membrane, network, etc. The nuclei appear to be of very variable size.

Now I am convinced that no series of vegetative changes in the nucleus such as Gonder describes really occurs. The appearances described—and very beautifully figured by Gonder—have, I believe, been wrongly interpreted.

In the living animal it is almost impossible to make out anything of the nuclear apparatus with certainty. It is, therefore, necessary to work chiefly on fixed and stained material. Unfortunately, the animals survive but a short time after removal from their host, no matter what precautions one takes. It is also a necessity, therefore, to fix the creatures immediately after removal. Moreover, if the host be allowed to die the parasites very quickly begin to

degenerate. In order to obtain satisfactory material I accordingly made preparations from the kidneys of the cuttlefish while still alive, fixing the smears, etc., as quickly as possible. When this is done the results are practically always the same after a reliable method of treatment. Excellent fixation can be obtained with any of the good fixatives in ordinary use—sublimite-alcohol (hot), picro-acetic (hot), and Hermann's solution being particularly good, especially the two former. The usual stains all give excellent results—even the very simplest giving quite exceptionally good pictures. I have found Delafield's hæmatoxylin and borax carmine (Grenacher) as good as anything one could desire. I used both moist film preparations—made by smearing the kidneys on a coverslip—sections, and the following method:—A small piece of the kidneys, containing many parasites, was fixed, stained entire, and finally teased up in clove oil. Isolated individuals could be examined in this way with great ease, though moist film preparations are perhaps the best. And the results at which I arrived were these. There is a nucleus constantly present in the form of a delicate network of chromatin and plastin. At no period in the living animal does it undergo a cycle of changes as described by Gonder. In addition to the network there are also to be seen in the cytoplasm—in greater or less numbers—particles which stain strongly with chromatin stains. (Cf. fig. 2.) From observations on a large number of organisms I am now convinced that this represents the normal condition of the nuclear material.

It now remains to answer the questions, "What are the chromatin particles in the cytoplasm?" and "What are the curious chromidial stages described by Gonder?"

Regarding the former, I think it may be regarded as certain that the chromatin particles are—in part, at any rate—ingested food material. As I have already shown, the majority of individuals—all those, in fact, which have attained any size—possess a mouth. And this very obviously serves for the ingestion of food, which appears to be largely com-

posed of the epithelial cells of the kidneys of the host. We do, indeed, see remains of cells in all stages of digestion (cf. fig. 8), and a careful examination of many different individuals has brought me to the conclusion that the majority of the chromatin particles in Chromidina are the remains of the nuclei of renal cells.

These ingested particles may be very strikingly demonstrated by staining the animal with neutral red intravital (fig. 3). The nuclear net remains unstained.

It is possible that the chromatin particles also constitute, in part, the micronucleus of the infusorian—the network representing the meganucleus. Multiple micronuclei are known in other Infusoria—e.g. in *Loxodes*, (cf. Joseph, 11).

Regarding the second question, I think there can be but little doubt that all the animals which show irregular lumps or granules of chromatin, in place of the delicate nuclear network, are abnormal. The appearances are caused by imperfect fixation. Almost immediately the animal dies, or is allowed to dry ever so little, the network breaks up, and its parts run together to form irregular chromatin masses. This can be easily proved by merely letting a smear preparation dry slightly in the air before fixation. The granular masses of chromatin then appear in nearly every individual in the preparation, after fixing and staining (cf. fig. 7).

Even in a well-preserved specimen it is often impossible to find the chromatin of the nuclear net continuous—because the distribution of the chromatin in the plastin network, which forms the basis of the nuclear apparatus, is not uniform. This is especially obvious in specimens which have been treated by a method involving differentiation after staining—e.g. iron-hæmatoxylin or borax carmine. The smaller masses of chromatin become decolorised before the larger—which apparently lie freely in the cytoplasm, though really imbedded in the plastin network (see fig. 1).

It is surprising that the nuclear apparatus in the head of the organism—when of large size—should have passed unnoticed. It is a most striking structure in the form of a

huge sling (fig. 6). Its gradual development from the simple network in a young "bud" can easily be traced (figs. 4, 5, 2, 6). The sling is seen to be composed of a number of parallel fibrils of plastin with chromatin granules imbedded in them (fig. 6).

In the process of segmentation ("budding") the nuclear net remains unaltered—from beginning to end of the process. This is well seen in fig. 1, where every stage in segmentation can be seen. Large segments are at first constricted off, and these subsequently divide in two.

I have never found individuals with the perfect "bladder" nuclei described by Gonder. Perhaps they are really the nuclei of the renal epithelium cells, either lying on the organism or after being ingested. The great size variation represented in Gonder's figure (Pl. 11, fig. 58) is worthy of note. I am inclined to think—after examining a large number of individuals—that they are not of normal occurrence during the vegetative life of the organism. But it is impossible to judge on negative evidence alone.

Chromidina coronata, Føett. emend. Gonder.

This infusorian differs from the preceding in the single character already observed by Føettinger and Gonder—the possession of a ring of long cilia surrounding the head, crownwise (fig. 8). The nuclear apparatus is exactly like that of *C. elegans* in every particular. The remarkable sling in the network in the head is just the same, and is found strongly developed in large individuals only (fig. 8).

Reproduction takes place in a manner exactly like that seen in *C. elegans*.

Opalinopsis sepiolæ, Føett.

O. sepiolæ differs considerably from the two infusorian parasites already considered. It has been described in some detail already, but the following points may be added to these descriptions (6, 7).

The vacuole, which is situated at the posterior end of the animal (fig. 9) is contractile. It pulsates at an average rate of about once a minute. It is one of the most characteristic features of the organism, and it is surprising that its contractions have not been remarked before. Most of the individuals which I observed contained crystalline bodies in their cytoplasm (fig. 9). There is no cystostome.

Although I succeeded in discovering but a single cuttlefish infected with this parasite, I was able to make a considerable number of observations upon it. For *Opalinopsis* survives, in carefully made preparations, for several hours after removal from its host, and continues to divide actively, thereby presenting a great contrast to *Chromidina*. The liver and kidneys of the infected *Sepia* were literally swarming with the parasites.

Very little regarding the nuclear apparatus can be made out in the living animal. My description is therefore based upon permanent preparations, made with the same precautions as those of *Chromidina*. And here again, I cannot agree with Gonder's interpretation of the appearances presented.

Foettinger found that "the nuclei . . . sometimes assume the form of a network, and all stages are to be found intermediate between these networks and scattered nuclei—spherical or rod-like" (6, p. 373).

Gonder believed that the changes seen in the nuclear apparatus were intimately connected with the division of the organism. The cycle of changes is as follows:—"1. A complete resolution and fragmentation of the lumps and particles into fine granules . . . 2. Division of the Infusoria; the animals attain their greatest size at the stage of complete resolution of the nuclear substances, whereupon they divide. 3. A reconstitution of the nuclear masses, i. e. the plastin collects itself at certain places in the walls of the alveoli, together with the granules—so that fragments arise which branch out into large bands and slings, out of which the nuclear masses—with which we started—are formed" (7, p. 254). All these stages are very accurately figured, and it

is from Gonder's interpretation of them only that I am compelled to differ.

I have found that when the animal is properly fixed and stained, the nucleus invariably has the appearance shown in figs. 10 and 11. That is to say, it forms a complete network of chromatin and plastin, lying in the cell—just like the nuclear net of *Chromidina*. The net is not always quite easy to make out in its entirety, owing to the manner in which the chromatin may be distributed in the plastin framework. Hence, when only a chromatin stain is employed, parts of the nucleus may appear detached (fig. 10). The size and complexity of the net vary a good deal. It often has a quite simple structure—especially in small individuals (figs. 12, 13).

Just as in *Chromidina* the network remains as such during division. All stages, from the very beginning (fig. 14) right up to the completion of the process of transverse division (fig. 15) are to be found. Division takes place rather rapidly—the organisms which I saw dividing taking about twenty minutes for the whole act.

Here again, as in *Chromidina*, the organisms which contain lumps and scattered fragments of chromatin are produced by imperfect fixation. The lumps appear as soon as the animals begin to die (figs. 17—19), and may take very different forms. The degeneration is also seen, as a rule, in the cytoplasm, which becomes more coarsely alveolar. This was noticed by Gonder, though he failed to realise its meaning. "The alveolar system changes its character with the nuclear changes. If the nucleus is broken up or completely fragmented—forming a chromidial apparatus—then the protoplasm has a coarsely alveolar structure" (p. 246). Gonder's figures show this very accurately (e. g. figs. 19, 20, 26, etc.). A condition in which the chromatin is completely dissolved in the cytoplasm (Gonder's fig. 19) has never come under my observation. It appears to me to be highly abnormal.

Although I have examined a large number of individuals of all sizes, and at all different stages of division, I have

never found any which contained a single nucleus, as figured by Gonder. I thought at one time that I had done so, but later I was able to prove that the large uninucleate cells (fig. 16) which I mistook for Infusoria in the preparation were really giant amœboid cells from the cuttlefish's kidney. Some of these cells attain a length of nearly $50\ \mu$.

Neither in Chromidina nor Opalinopsis has any sign of conjugation been observed.¹ No sexual process of any sort is known.

Equally unknown is the method of dissemination in nature. No cysts or resting stages have ever been seen. It is a curious fact that—like their frequent companions, the dicymids—the Infusoria are unable to live for more than a few minutes in sea water. How they reach their host is still a mystery.

I should like to correct here the statement made by Gonder (7, p. 246) that the colour of the liver is an index of infection. As a matter of fact, the liver in a perfectly fresh uninfected cuttlefish varies in colour from dark red-brown up to creamy white, apparently according to the relative amount of cellular and non-cellular substance which it contains. In livers of very pale colour, only a few shreds of living tissue are to be found. Colour seems dependent mainly upon metabolism, not parasites, though these might, of course, affect it occasionally to some extent.

The significance of the nuclear apparatus.

A comparative study of the nuclear apparatus of Chromidina and Opalinopsis brings some interesting points to light. I will here indicate a few of these.

As I have already shown, in both Chromidina and Opalinopsis the nuclear apparatus consists of a delicate network, composed of chromatin granules imbedded in a

¹ The figure given by Fœttinger (6) showing "conjugation" in Opalinopsis is, as Gonder justly remarks, nothing more than a stage in division.

plastin matrix, which extends through the cell. This network represents the compact nucleus which we are accustomed to see in other organisms. To speak of it as a "chromidial net," as does Gonder, is, to my mind, misleading. For there is absolutely no indication that it is in any way comparable to the structure known as a chromidial net in *Thalamophora*, etc. It is merely a modification of the branched form of nucleus.

The branching type of nucleus has been long familiar to cytologists. It is well seen in the cells of certain insects, as we know from the work of the Hertwigs, Brant, Eimer, Balbiani, etc. (Cf. R. Hertwig's description (10) of the "amœboid" nuclei in the Malpighian tubule cells of *Pieris brassicæ*.) But the most instructive comparisons are to be made with the nuclear apparatus of other Infusoria.

Maupas (12), Gruber (8, 9), and others have described various forms of diffuse nucleus in the Infusoria. One of the most careful descriptions is that by Gruber (9) of the hypotrichous ciliate *Holosticha* (*Oxytricha*) *scutellum*, Cohn. In this organism both meganucleus and micronucleus lie scattered in fragments through the cytoplasm during vegetative existence. Before division, however, the fragments come together, forming a single mega- and micronucleus, both of which then divide, subsequently fragmenting once more in the daughter individuals. This formation of a compact nucleus before division does not appear to take place in all "multinucleate" forms, e.g. *Loxodes*. In *Trachelocerca*, *Uroleptus*, and *Epiclinites* also the nucleus is diffuse (Gruber, 9).

It is in the parasitic Infusoria, however, that the most interesting forms for comparison with Chromidina and Opalinopsis are to be found. In *Fættingeria actiniarum*, Clap.,¹ a nuclear apparatus very like that of *Opalinopsis* has been described by Caullery and Mesnil (3). In

¹ = *Fættingeria* (*Plagiotoma*, *Conchophthirius*) *actiniarum*, Claparède emend. Caullery et Mesnil. The animal lives in the cœlenteron of various sea-anemones.

the youngest animals, the nucleus is roughly horse-shoe shaped, but in large individuals it takes the form of a mesh-work of chromatin containing nucleoli at certain points. The network, which varies in its form, is described as consisting of a system of "tubes," and as being "amœboid." It bears, as Caullery and Mesnil have pointed out, a very striking resemblance to the nuclear apparatus, as I have seen it, in *Opalinopsis*.

But the most interesting comparisons are to be made with various *Anoplophryinæ*. Recent research has brought to light many interesting facts regarding this group. As is well known, in *Anoplophrya* the nucleus is band-like, running down the middle of the body of the elongate organism. The animals possess a series of vacuoles and a method of segmentation which resemble the conditions seen in *Chromidina* to a remarkable degree. But at first sight the nucleus appears totally unlike. The means of comparison have been given us by Caullery and Mesnil (4), who have discovered a remarkable new member of the group—*Rhizokaryum concavum*, C. et M. In this animal—a parasite of certain species of *Polydora*—there is a nucleus consisting of a thick axis, from which numerous branching processes are given off ("like a root with numerous rootlets"). According to these observers, in *Anoplophrya* also the central nuclear cylinder sometimes shows little pointed appendages, thus presenting an appearance intermediate between a simple band and a branching stem like that of *Rhizokaryum*. From the latter condition it is not difficult to imagine how a reticular nucleus like that of *Chromidina* might have arisen from an originally compact nucleus. The last barrier between the infusorian parasites of cuttlefish and the *Anoplophryinæ* has now been broken. And it is certain, as Neresheimer (13) hinted from his study of *Opalina*, that the parasites from cephalopods are not related to *Opalina* but to *Anoplophrya*.

One or two other points of interest may be briefly touched upon. The most interesting is the apparent absence of a

miconucleus in the parasites of cephalopods. Nor is a miconucleus described in *Fœttingeria*. In *Rhizokaryum* the miconucleus is spindle shaped. Some very interesting observations have recently been made upon a form very closely allied to *Anoplophrya* by Awerinzew (1). He names this animal (a parasite of the marine worm, *Ophelia limacina*) *Bütschliella opheliæ*; and he finds that the miconucleus becomes visible only when the animal is about to divide. In *Chromidina*, however, a miconucleus is never visible at any stage during segmentation (cf. fig. 1).

As I have already pointed out, the chromatin particles, which are normally present in the cytoplasm, may in part represent the miconucleus. A curious formation, apparently from the nucleus, of similar particles occurs at a certain stage in *Bütschliella*. Another interesting feature of this organism is that it may undergo a simple transverse fission, thus combining both the method of reproduction seen in *Chromidina* and that of *Opalinopsis*. *Bütschliella* also possesses contractile vacuoles.

Of the deeper significance of the net-like nucleus we know nothing. It is as yet quite impossible to say why one organism should possess a single compact nucleus, whilst others of similar size and apparently performing similar functions should have nuclei in the form of a net or scattered fragments. It looks at present as though it were immaterial how the nuclear substances are disposed in the cell so long as they are present. However, the matter can be elucidated by further research alone.

The foregoing pages embody a small part of the results of the work which I did whilst occupying the British Association Table at Naples from March to June of the present year. I desire to thank the British Association Committee for their kindness in assigning me the Table. I wish also to tender

my warmest thanks to the Goldsmiths' Company for their grant, without which I should not have been able to carry out my work in Naples. I trust the remaining results will be ready for publication before long.

CAMBRIDGE;

August, 1908.

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EXPLANATION OF PLATE 1,

Illustrating Mr. C. Clifford Dobell's paper on “Some Observations on the Infusoria Parasitic in Cephalopoda.”

FIG. 1.—*Chromidina elegans*, posterior end, showing various stages in the formation of buds. The chromatin is alike at all stages. (Hot picro-acetic, borax-carmin, differentiated acid-alcohol. Leitz $\frac{1}{12}$ in. \times 1.)

FIG. 2.—*C. elegans*. Medium sized individual, entire. Note the nuclear network, chromatin granules, mouth, etc. (Sublimate-alcohol (hot), Delafield's hæmatox. $\frac{1}{12}$ in. \times 1.)

FIG. 3.—*C. elegans*. Living animal: stained intravital with neutral red. ($\frac{1}{6}$ in. \times 5.) The food particles, vacuoles, and mouth are well seen.

FIGS. 4, 5, 6.—Three stages in the development of the head of *C. elegans*. 4. A small individual, without a mouth. 5. A larger animal, with a small mouth and feebly developed chromatin sling. 6. Very large individual, with well developed mouth and strongly developed sling. ($\frac{1}{12}$ in. \times 1 (enlarged to scale). Hot sublimate alcohol, Delafield.)

FIG. 7.—Posterior end of *C. elegans*, forming segments. The animal had died before fixation, thus giving rise to nucleus in the form of chromidia. (Sublimate alcohol, Delafield, $\frac{1}{12}$ in. \times 1.)

FIG. 8.—Head of *Chromidina coronata*, large individual. The chromatin sling is well seen (cf. fig. 6). *m* = position of mouth. *c* = an ingested cell from the kidneys. Various other cell remains are also to be seen. (Hot picro-acetic, borax-carmin. $\frac{1}{12}$ in. \times 1, enlarged.)

FIG. 9.—*Opalinopsis sepiolæ*. Ordinary individual, showing contractile vacuole (*c.v.*), crystalline bodies, cuticular striation, etc. Living animal. ($\frac{1}{12}$ in. \times 1.)

FIGS. 10, 11.—*O. sepiolæ*, stained, showing nuclear network. Large individuals. (Sublimate alcohol, Delafield. $\frac{1}{12}$ in. \times 1.)

FIGS. 12, 13.—Two small *O. sepiolæ*. (Sublimate alcohol, Delafield. $\frac{1}{12}$ in. \times 1.)

FIG. 14.—*O. sepiolæ*. A large individual beginning to divide. Note persistence of nuclear net. (Sublimate alcohol, Mayer's paracarmine. $\frac{1}{12}$ in. \times 1.)

FIG. 15.—*O. sepiolæ*, at end of division. The nuclei are still in the form of a net. (Sublimate alcohol, Delafield. $\frac{1}{12}$ in. \times 1.)

FIG. 16.—Giant amœboid cell from liver of *Sepia officinalis* infected with *Opalinopsis*. Length 43 μ . (Sublimate alcohol, Delafield. $\frac{1}{12}$ in. \times 1.)

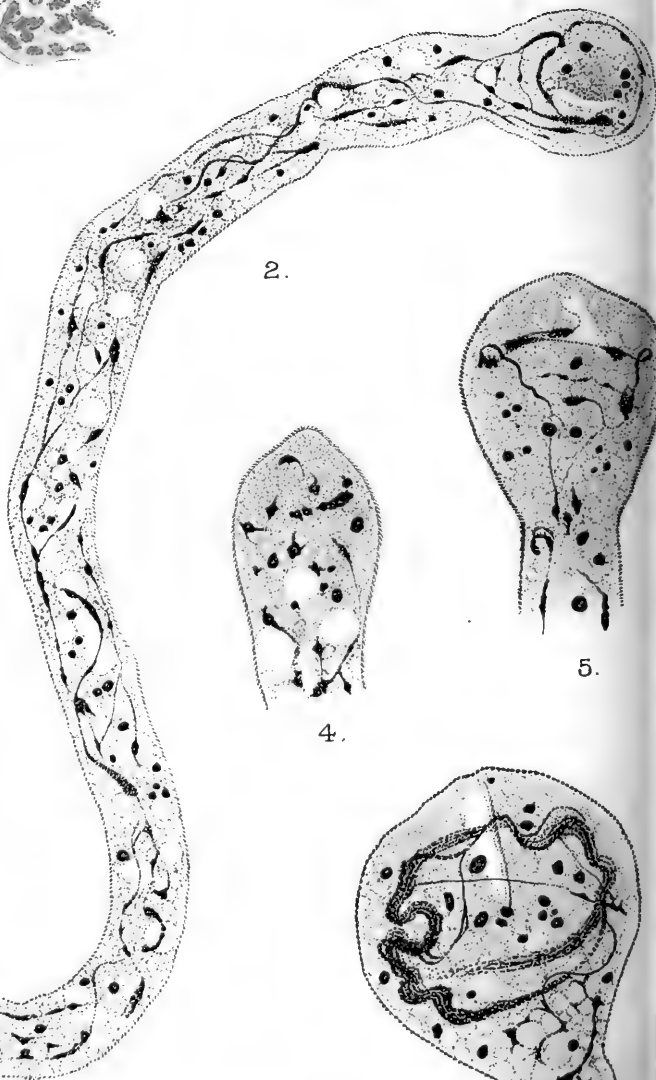
FIGS. 17, 18, 19.—Degenerate forms of *O. sepiolæ*, with fragmented nuclei. (Sublimate alcohol. 17, Delafield; 18, 19, Grenacher's alum-carmine. $\frac{1}{12}$ in. \times 1.)

[With the exception of figs. 3, 8 (in part), and 9, the cilia are not shown.]

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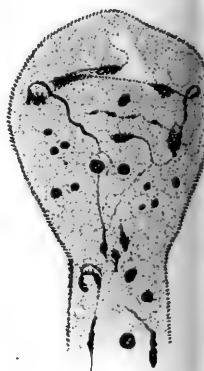
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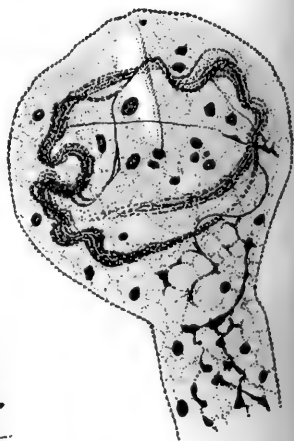
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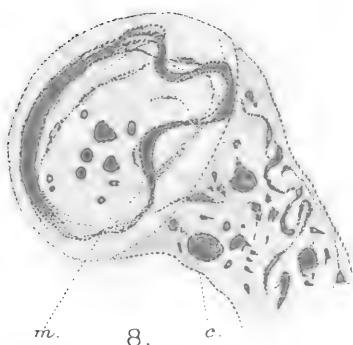


7.





3.



m.

8.

c.



9.

c.v



10.



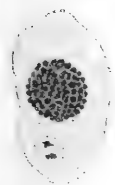
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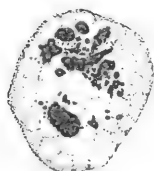
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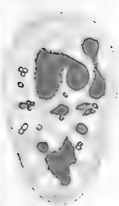
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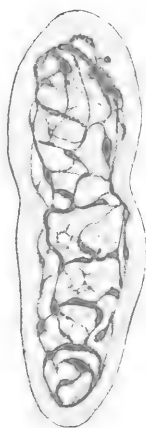
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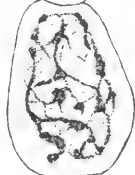
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19.



14.



15.



Researches on the Intestinal Protozoa of Frogs and Toads.

By

C. Clifford Dobell.

Fellow of Trinity College, Cambridge; Balfour Student in the University.

With 1 Text-figure (page 215) and Plates 2-5.

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INTRODUCTION.

THE observations recorded in the following pages are the results of an attempt to discover the life-histories of the protists which inhabit the gut of the common frog and toad. My original intention was the investigation of the life-history of *Trichomonas*. But so great is the number of organisms which live in company with this form that I very soon perceived that it would be almost impossible to confine my investigations to a single species. Every organic particle in the alimentary canal had to be tested regarding its possible relationship to the organism which, in particular, I was examining. For example, I not infrequently found a number of cysts occurring side by side with *Trichomonas* in the frog's gut. To connect the two, without further evidence, would be ridiculous. I therefore had to discover to what organism the cysts belonged. And thus, time after time, I found myself driven to determine, to the best of my ability, the life-histories of all the protists which I encountered. The number of these is considerable. Therefore there were many difficulties in the way of success, and therefore, also, my work remains still far from finished.

My attention from the first has been chiefly directed towards the smaller protists, as these were relatively less known. There is but one of the larger forms, however—*Opalina*—which has really been carefully studied.

A part of my work has already been published—namely, that dealing with the little flagellate which I have called *Copromonas subtilis* (10); that dealing very briefly with a very small portion of the bacteria-like organisms (11); a preliminary notice of the organisms discussed in the present paper (12); a description of a portion of the life-history of the yeasts (13). I have also published some observations on a peculiar process of degeneration in *Opalina* (9).

I will preface my own observations with a brief summary of the work which has previously been done by others.

HISTORIC.

I wish to give here only a general—and very brief—synopsis of the work which has been done upon the protists in the gut of the frog. I shall have to consider the various organisms in greater detail later, as I come to them. The subject is one of some interest, however, for it engaged the attention of some of the earliest microscopists.

Probably the first man to discover the existence of Protozoa in the intestine of the frog was van Leeuwenhoek, who, in 1683 ('*Omnia Opera*'), described and figured "animalcula in stercore Ranarum." These "animalcules" are generally supposed to have been *Opalina intestinalis* Ehrbg. Later, Leeuwenhoek carried his researches further, and was able—in 1702—to recognise three different protozoan "animalcula in the excrements of frogs." The species were, in all probability, *Nyctotherus cordiformis* Ehrbg., *Opalina intestinalis* Ehrbg., and another organism which was probably *Trichomonas* or *Trichomastix*—"Bodo ranarum" according to Ehrenberg.

For more than a century subsequently the subject received only brief and occasional notice. But I may mention during this period the names of Bloch (1782) and Göze (1782) who both devoted themselves—more or less successfully—to the study of these "intestinal worms" (*Opalina*, etc.). It was not until 1838 that any considerable advance was made. In this year—a landmark in the history of protistology—appeared the great work of Ehrenberg (16). Here we find that the author was able to distinguish no less than eight different species of protists. I give these below, with their probable synonyms in use at the present day:

1. *Bodo ranarum* Ehrenberg . = *Trichomonas* or *Trichomastix*.
2. *Bodo intestinalis* Ehrenberg . = *Octomitus*.
3. *Bursaria ranarum* Ehrenberg . = *Opalina ranarum*.
4. *Bursaria intestinalis* Ehrenberg . . . = *Opalina intestinalis*.

5. *Bursaria* (?) *cordiformis* Ehren-
 berg. = *Nyctotherus cordiformis*.
 6. *Bursaria* *nucleus* Ehrenberg } = *Balantidium entozoon*.
 7. *Bursaria* *entozoon* Ehrenberg }
 8. *Vibrio* *bacillus* O. F. M. . = ?

Though necessarily imperfect, and in many cases highly fantastic, the descriptions of Ehrenberg remain in many ways a model of accurate and careful observation.

From the time of Ehrenberg down to the present day this little group of protists has received—with one exception—but scant attention. The exception is *Opalina*, of whose life-story, owing to the admirable researches of Zeller, Neresheimer (40),¹ Metcalf, and others, we now have a fairly perfect knowledge. As for the remainder, so little of the life-history has been discovered hitherto—the observations on them being mainly published in the form of short notes—that I will not discuss them further here.

MATERIAL AND METHODS.

The methods employed in the following researches have been, for the most part, the same as those which I have already described in a previous paper (10), to which the reader is referred. I will here add only a few remarks regarding one or two special points.

The frogs and toads have all been obtained either in Cambridge or in Munich. I have worked upon *Rana temporaria* L., *R. esculenta* L., and *Bufo vulgaris* L. As I have carried out the researches in two different laboratories the optical apparatus employed has been somewhat varied. But that has made no difference of any importance. The objectives, etc., employed will be found in the explanation of the figures, likewise the technique for fixing and staining. I may add, however, that the best fixation has always been obtained with Schaudinn's sublimate-alcohol, and the best staining with Heidenhain's iron-alum hæmatoxylin or Dela-

¹ A complete list of the literature on *Opalina* will be found appended to the work of this investigator.

field's hæmatoxylin. But I have used all the ordinary fixing fluids and stains. The greatest importance has always been attached to observations on the living animal.

As the organisms described naturally live in an anaërobic condition they are most suitably examined under tightly waxed-down cover-slips, and not in hanging-drop preparations.

It is almost impossible to obtain the contents of the frog's alimentary canal when required whilst the animal remains alive. Therefore I have had to resort to various means for obtaining the necessary material. I have frequently used the following method: A frog is taken and its brain (but not its spinal cord) pithed. When it has recovered from shock it is, of course, still quite lively, and will live for a long time. I pin the animal down in a dissecting dish, and by making an incision into the abdomen remove the contents of the large intestine by operating directly on it. If the frog be kept cool it will live for many days, thus enabling one to go on removing the gut contents at any required intervals of time. I have found the most suitable method of extracting the contents of the large intestine is to make a small cut into the small intestine at its juncture with the large. The contents of the large intestine can then be removed through the hole, and when sufficient has been extracted a piece of cotton can be tied immediately below the incision so as to close the large intestine once more. The frogs must be kept damp by covering them with wet cloths.

So many different organisms are to be found in the intestine of the frog¹ and toad that it will not be out of place to refer to these briefly at this point. In addition to the animals described in detail in subsequent pages, there are the following:

Among Protozoa we find *Opalina ranarum* Purk. et Val., *Nyctotherus cordiformis* Ehrenberg, *Balantidium entozoon* Ehrenberg, *Balantidium duodeni* Stein, and *Balantidium elongatum* Stein.² *Copromonas* is occa-

¹ *Rana temporaria* L. has been especially studied.

² First recorded by Dale (6).

sionally present. Of Bacteria there is an immense number of species, for the most part undetermined (cf. 11), belonging to the genera *Bacillus*, *Micrococcus*, *Spirillum*, *Sarcina*, etc. Several species of yeast (cf. 13) are also commonly to be found. And there are several different other fungi, the most remarkable of which is *Basidiobolus ranarum* Eidam. The cysts of this organism are common, and might be mistaken for those of *Chlamydomyces* or *Copromonas*, though they are usually a good deal larger. Other developmental stages of this very interesting fungus are also quite often encountered, as the cysts germinate in the faeces. Then the metazoan parasites must be mentioned. These are worms of different sorts—trematodes (*Distomum*, etc.), nematodes (*Strongylus*, *Oxysoma*, etc.), and an occasional cestode (*Tænia dispar*). The eggs of these forms—especially those of nematodes—are also usually to be found in abundance. The other organic particles which one encounters are chiefly degenerating epithelium cells and blood-corpuscles. Then, in addition, there are all the thousand and one undigested animal remains of the host's diet—remains of insects, bits of chitin, setæ of earthworms, fat droplets, etc.—together with shells of diatoms and desmids. I have also found the unopened and apparently intact spores of *Monocystis*¹ (from earthworms—several species) and *Adelea ovata* (from centipedes). Very many inorganic particles—e.g. various crystals, sand grains, etc.—are, of course, also present in greater or less numbers.

I will now proceed to the detailed description of the organisms whose life histories have especially engaged my attention.

A. FLAGELLATA.

(1) The Trichomonads.

It has hitherto been universally supposed that but one trichomonad occurs in frogs, namely *Trichomonas*

¹ First noticed, I believe, by Lieberkühn (1854).

batrachorum Perty. There are, however, in reality two, a *Trichomonas* and a *Trichomastix*. I will begin with the latter.

(a) *Trichomastix batrachorum* Dobell.

I have already described this organism in my preliminary note (12). It differs structurally but little from other species, and is very common. It may occur alone, but is more commonly found in company with *Trichomonas*.

Structure.—In all essential points this animal's structure is identical with that of *Trichomastix serpentis*, which I have elsewhere described (56).

The general external form is usually ovate or pyriform, but subject to a certain amount of modification (see Pl. 2, figs. 1-3). The nucleus lies at the anterior end of the body, and is ovoid and composed of chromatin granules of irregular size and shape. A nuclear membrane is usually seen. Lying in front of the nucleus and generally in close apposition is a minute granule which stains with chromatin stains very intensely. This granule is often seen to be really double (cf. figs. 1, 3), and it serves as the point of origin of the four flagella. For reasons which will be apparent later I shall call this little diplosomic structure the blepharoplast.¹

Of the flagella, three are directed forwards whilst the fourth is turned backwards (cf. fig. 3, etc.).

The flagella are not the only organellæ which find an attachment to the blepharoplast. A flexible rod-like organ is also firmly fixed to it, and runs backwards to end in the caudal process of the animal. This organ is one of the most characteristic features of the trichomonads, and although it has often been observed in *Trichomonas*, its significance has not always been properly understood. Its real function is undoubtedly skeletal. It serves as a fixed point for the anchorage of the flagella. Since the structure is one which

¹ The name was first used for trichomonads by Laveran and Mesnil (65).

occurs in more than one flagellate which I shall have to describe, and since no convenient name has yet been given to it, I propose to call it the axostyle¹—a name which I think suitably describes it.

As in *T. serpentis*, the axostyle goes either through or over the nucleus to reach the blepharoplast. There can be little doubt that blepharoplast and axostyle are really united. That this is so is seen especially clearly in some cases where the end of the axostyle is bent (cf. fig. 2). I mention this because it has not been clearly made out by most investigators, e.g. by Prowazek in *T. lacertæ*.² The thickness of the axostyle is very variable. Two distinct types of organism can be thereby distinguished—a type with a very slender axostyle (fig. 3), and a type with a very thick one (fig. 2). Intermediate forms occur, but most of the animals can be classified under one type or the other. In the forms with a slender axostyle, one very frequently finds a few very intensely staining granules immediately above the point where it enters the caudal process (fig. 3). Their meaning is obscure.

A well-marked cytostome is usually to be seen (fig. 1).

The largest forms reach a length of 20 μ , from extreme anterior end to tip of axostyle. Very minute forms, about 6 μ long, are also found occasionally, but the average length is about 15 μ .

Although there is no visible cuticle, the animal does not exhibit as a rule any irregularity of contour. Only when it degenerates does it become amœboid. The cytoplasm is generally filled with food bodies.

The movements resemble those of *Trichomonas*, which have been often enough observed.

I have found the creature in *Rana temporaria*, but never

¹ The terms used by other writers are not few, and are mostly descriptions rather than names—e. g. "axial rod," "pointed organ," "baguette squelettique," "baguette interne," "style hyalin," "côte," "Achsenstab," "Rückenleiste," "Kiel," "Rippe," "costa," "bastoncello assile," etc.

² I have myself seen the attachment very clearly in this form on several occasions.

in *R. esculenta* or *Bufo*. It is less frequently present than *Trichomonas*.

Division.—Stages in division are very difficult to find. I had examined a very large number of living animals and had made over two hundred moist film preparations before I hit upon a single stage. When present they usually occur together. By making a large number of preparations I have been able to find practically every phase of division, though my observations on the living organisms have been fragmentary. I have not succeeded in following out the entire process from beginning to end in one and the same animal.

Division is longitudinal and takes place as follows (see Pl. 2, figs. 4—12): The first thing to be seen is that the axostyle and nuclear membrane vanish, being apparently absorbed, so that a form like that shown in fig. 4 is produced. The chromatin lies freely in the neighbourhood of the blepharoplast in the form of small granules of varying sizes. Even at this stage (fig. 4) it can usually be seen that the blepharoplast itself is becoming elongated, assuming a dumb-bell shape. It then becomes drawn out to such an extent that it takes on the appearance of a little rod. Two flagella remain at either end of this rodlet¹ (fig. 5). It appears to me that of the two granules which normally make up the blepharoplast one bears the posterior flagellum and the other bears the three anterior (cf. fig. 4). But during the division of the blepharoplast to form the rod one anterior flagellum in some way migrates over to the posterior flagellum, so that two flagella come to lie at either end of the rod (fig. 5). Next, the ends of the rodlet show an enlargement, so that the whole of the structure derived from the blepharoplast assumes the appearance of a very attenuate dumbbell (fig. 6). At the same time the chromatin granules, which were previously lying in a small indefinite heap, arrange themselves in the

¹ The origin of the flagella is not always made out with ease. For, owing to the way in which they get curled up, superimposed and entangled, they present appearances which at first sight are frequently very deceptive.

form of a spindle round the rodlet (fig. 6). I have never been able to make out achromatic spindle fibres at this stage (cf. *Trichomonas*, p. 217, and fig. 21).

At this stage, or perhaps earlier, the new flagella begin to make their appearance. They grow out from the thickened ends of the rodlet—which, from their subsequent development, I shall now call the daughter blepharoplasts—as four (i. e. two from each blepharoplast) small peg-like structures, which are easily recognised in Heidenhain preparations by their greater thickness and more intense staining. They do not always appear simultaneously (see figs. 7, 8, etc.). One now notices that the chromatin masses itself together into a few large, irregular, very strongly-staining lumps, which lie near the centre of the rodlet (fig. 7). The number of these masses varies, and they are usually difficult to count with accuracy. About six are present. They cannot justly be called chromosomes. It seems that the organism remains in this condition for some time, for it is the stage which is by far the most frequently encountered in stained preparations.

In a little while the chromatin heap becomes divided in two and each half travels along the rod, uniting the daughter-blepharoplasts, to take up a position by them (figs. 8, 9). The arrangement of the rod, blepharoplasts, chromatin masses and young flagellar outgrowths is particularly well seen in the specimen shown in fig. 9.

When it has reached the region of the blepharoplast each chromatin mass fragments and constitutes a new nucleus (fig. 10). During this process the rod becomes thicker and begins to stain less intensely (fig. 10). Hand in hand with the nuclear changes have gone changes also in the configuration of the cytoplasm. Whilst this was originally of a somewhat oval contour (figs. 4, 5), it passed through a stage of being roughly triangular (figs. 8, 9, etc.) to the present condition, which is more or less reniform in outline (fig. 10).

For a long time I was unable to find any further stage than this in my permanent preparations, although I searched long and carefully. The reason for this I then discovered from

observing the living animal. After this stage the animal completes its division with great suddenness. After remaining for some time in the state shown in fig. 10 a kind of constriction appears very suddenly in the middle (fig. 11). The constriction deepens all of a sudden, and then almost disappears again, appearing as though an unseen string were suddenly tightened and then loosened around the animal. This welling in and out lasts for several seconds, being repeated some half-dozen times, and then in a flash the creature is snapped in two by the constriction being completed, and two little daughter monads are left facing in opposite directions (fig. 12). For several seconds they remain thus, moving their flagella but feebly. Then they become more active by degrees and swim away from one another. It is seen that each monad possesses all the organellæ of the adult, and it is also perfectly plain that the rod which united the daughter blepharoplasts has, by dividing transversely, furnished each daughter monad with its axostyle. The axostyle is thus re-formed by the blepharoplast at each division. I will discuss the interesting points connected with this later (see p. 225).

The behaviour of the cytostome is not easy to make out during division. Very often, however, it can be quite clearly seen that the cytostome passes over into one of the daughter individuals (cf. fig. 10), so that the other individual must generate a new mouth. This is in agreement with Prowazek's observations on *T. lacertæ* (73).

Encystment.—After continuing to divide for an unknown length of time *Trichomastix batrachorum* is able to encyst. For a long time I was quite unable to find any trace of encysting in this animal. Even now I have not the remotest idea what causes encystment. In the ordinary course of events the animals, whether liberated in the fæces or removed by operation from the host, die sooner or later. And this happens no matter how they are treated—whether allowed to dry, whether placed in water, whether kept moistened in the fæces. All experiments to determine the cause of cyst-

formation have been negative. Neither change of temperature nor nutrition of the host appears to have the slightest influence. When I had almost despaired of ever finding the cysts I suddenly came upon them—in apparently quite ordinary frogs. It is curious—though perhaps a mere coincidence—that the cysts were all found in the months of November, December and January, before, and in part contemporary with, the period of cyst-formation in *Opalina*. When the cysts are present they are usually found in fairly large numbers, for many animals encyst at the same time.

Before encysting the animal undergoes considerable changes as regards its nuclear structure. Instead of the chromatin remaining distributed in the form of fine granules throughout, it begins to concentrate in the centre and in the nuclear membrane. The result is the formation of a nucleus with a sharp chromatic outline and a very distinct karyosome (fig. 13). A delicate thread is usually to be made out running in a longitudinal direction and uniting the karyosome with the membrane above and below (cf. fig. 13).

When the animal has reached this stage it begins to round itself off and decrease in size, preparatory to secreting a cyst wall. This process takes a very long time, so that it is almost impossible to follow it out in one and the same animal. However, I have seen every stage in different animals so many times that there can be no doubt about what occurs. The first thing that happens is that the axostyle begins to disappear, gradually dwindling away from behind forwards. As the caudal process ceases to exist the animal is able to round itself off. It does so, coming slowly to rest. After a time the movements of the flagella get slower and slower, and finally cease. Then the flagella disappear. They seem to dissolve, but it is difficult to see what becomes of them. It is just possible that they are drawn into the body, as in *Copromonas* in division. The blepharoplast remains behind, lying upon the nucleus (fig. 14). A diminution in size takes place, so that the organism shrinks to an oval mass of protoplasm. In this stage it forms the cyst membrane (fig. 14).

At first this is soft, but later it becomes harder and thicker. The axostyle gradually goes, and during the time of its disappearance a little darkly-staining, triangular area is generally visible between its remains and the nucleus (fig. 14). The significance of this is not apparent.

In the end the axostyle completely vanishes. The nucleus becomes drawn out in its long axis to such an extent that it often comes to stretch almost from one end of the cyst to the other (fig. 15). The karyosome also shares in the process, becoming drawn out into a long strand, which remains united to the membrane at either end. Above the nucleus, and in contact with it, the karyosome can generally be seen as a minute diplosomic structure (fig. 15). This stage is the last, and the cysts must now be regarded as permanent structures, which probably serve for the dissemination of the parasite. Although I have had cysts under observation for weeks at a time they have never undergone any further change. This is not difficult to determine, because although very small their structure can be made out quite clearly—with proper illumination, etc.—in the living state.

The cysts vary in size from ca. 4μ – $7\mu \times$ ca. 4μ – 6μ , but average dimensions are ca. $6.5\mu \times$ ca. 5μ .

The reduction in size in course of encystment is probably brought about by loss of water. It seems likely that before the reduction begins an actual diminution in the amount of solids in the composition of the protoplasm takes place. On several occasions I found that the large animals which were about to encyst were extraordinarily hard to fix. Instead of fixing in the ordinary way they collapsed, leaving only a few shreds of protoplasm and nucleus behind. The smaller animals—those in later stages of encystment—were fixed quite well however.

I have many times endeavoured to cause the animals to leave their cysts again, by treating them with the digestive juices of the frog. But all attempts have failed—a fact which I attribute to the abnormal condition of the laboratory frog, more especially in winter, when the experiments were made.

(Cf. similar results obtained with the amœba and coccidia, p. 253, etc.)

According to Prowazek (73), the division of *Trichomastix lacertæ* differs from that which I have just described. It appears from his account that the axostyle is drawn up towards the nucleus and then rearranges itself at right angles to its original position—passing through a T-shaped phase in doing so. The connection of the axostyle to the blepharoplasts was not made out. When the rod is rearranged the nuclear chromatin travels in two masses to each of its ends. The axostyle thus appears to function as a kind of division centre. From my own observations on this organism I believe that its structure and method of dividing are identical with those just described in *T. batrachorum*. But unfortunately I have found only a very few stages in division, so that I may be wrong. Some of Prowazek's figures, however, also support my interpretation (cf. figs. 8, 10, Pl. 1 [73]).

The method of division which I have elsewhere described in *T. serpentis* (56) also differs considerably from that of *T. batrachorum*. As my observations were made chiefly on living organisms, it is possible that I misinterpreted what I saw. Nevertheless I was able to watch division many times with great clearness, and believe the figures and description I have given are substantially correct for the living animal. The presence of a filament connecting the blepharoplasts after division may, however, have escaped my notice.

Prowazek (73) has described an autogamy in the cysts of *T. lacertæ*, but I have never seen anything at all like it in *T. batrachorum*. The cysts of the former species seem to be totally different in every way.

(b) *Trichomonas batrachorum* Perty.

Syn.: [? *Bodo ranarum* Ehrenberg, 1838].

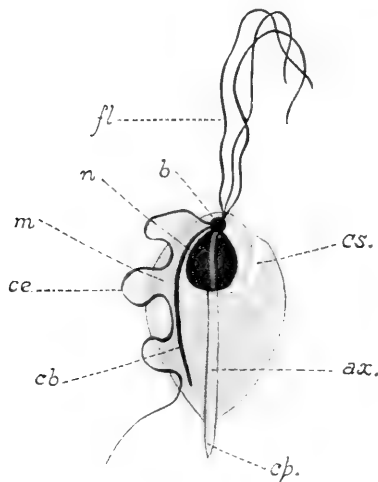
Monocercomonas batrachorum Grassi, 1879.

Cimænomonas batrachorum Grassi, 1882.¹

Trichomonas batrachorum (Perty) Stein, 1878; S. Kent, 1880; Bütschli, 1884; Blochmann, 1884; Doflein, 1901, etc.

This animal was first recognisably described and named by Perty in 1852. I retain Ehrenberg's emended spelling of

TEXT-FIG.



Trichomonas batrachorum—diagrammatic. *n.* nucleus; *b.* blepharoplast; *fl.* flagella; *ax.* axostyle; *cp.* its caudal process; *m.* undulating membrane; *ce.* chromatic edge of same; *cb.* its chromatic basis; *cs.* cytostome.

the generic name introduced by Donné (*Tricomonas*, 1837, for *T. vaginalis*).

The occurrence of the parasite differs somewhat from that of *Trichomastix*. It is found not only in *Rana tempor-*

¹ Grassi here gives the following synonyms: "*Cercomonas intestinalis* ? Ehrbg.," "*C. ranarum* ? Ehrbg.," *Bodo intestinalis* ? Ehrbg., and *Bodo ranarum* ? Ehrbg. The first pair are really synonyms for the second pair. (*Cercomonas* = Dujardin 1841, used to replace *Bodo* Ehrbg. by Perty 1852.) *Bodo intestinalis* Ehrbg. is probably *Octomitus* (see further on under this heading).

aria but also in *Rana esculenta* and *Bufo vulgaris*.¹ It is quite probable that *Trichomastix* really also occurs in the last two, though I have never as yet encountered it there.

Structure.—Now that I have described the anatomy of *Trichomastix* it will be an easy matter to describe *Trichomonas*, for the two animals are alike in most respects. The only notable difference is that *Trichomonas* possesses an undulating membrane in place of the posterior flagellum of *Trichomastix*. The structure of the animal is shown in the accompanying text-figure. It will only be necessary to say something in addition about the undulating membrane (see also Pl. 2, fig. 16).

The undulating membrane resembles that of a trypanosome. It has a well-differentiated thickened border, which ends posteriorly in a free flagellum, as in *Trypanosoma*. This edge stains very intensely with iron hæmatoxylin, and to a less extent with other chromatin stains. In addition to this, however, there is also a rod-like chromatic basal structure, whose extent and degree of development vary a good deal. Sometimes it is represented merely by a few granules, arranged in a moniliform manner (as in the lower of the two membranes in fig. 20). Both the chromatic edge and the chromatic basis take their origin in the blepharoplast.

The rest of the animal's organisation is the same as that of *Trichomastix* (cf. figs. 1 and 16, Pl. 2).

The undulating membrane during life moves like that of a trypanosome. It will be superfluous to describe its movements.

It is surprising that so much uncertainty should have existed regarding the structure of this organella. Grassi (21) described it as a flagellum, but later (22) allowed that it might be a flagellum united to the body so as to form a kind of membrane. The discrepancy probably arose from his having observed both *Trichomonas* and *Trichomastix*. Stein

¹ And in *Hyla arborea* (Grassi).

had previously described it (46) as a "der Bauchseite angehörige Reihe von spitzzackigen, undulirenden Fortsätzen, welche gewöhnlich für Wimpern angesehen wurden." This investigator also saw the axostyle and nucleus. Seligo (44) inclined to Stein's interpretation of the structure, but Blochmann (1) and others clearly recognised its true nature.

The method of division, excepting as regards the membrane, is almost identical with that of *Trichomastix* (see figs. 17—24). I will therefore merely note the few points of difference.

The undulating membrane appears to be multiplied by splitting. In this the chromatic border takes part, but not the chromatic basis (figs. 17, 23). The latter never seems to split, but seems to be absorbed and reformed in each daughter-membrane. But it is not easy to see what happens to it exactly. The two membranes may become completely separated at quite an early stage (figs. 18, 20), or may remain attached posteriorly till quite late (fig. 23).

The blepharoplast and axostyle behave in exactly the same way as in *Trichomastix* (cf. figs. 5—10). The stage figured in fig. 21 is specially instructive. There is a very distinct spindle figure, much more clearly marked than anything I have ever found in *Trichomastix*. Moreover, the resemblance of the blepharoplasts to centrosomes is particularly striking (see discussion of this matter, p. 220 et. seq.). In the animal here figured the double nature of the blepharoplasts was also particularly clearly shown.

I have not found so many division stages in *Trichomonas* as in *Trichomastix*, but the likeness between them is so great that I have little doubt that they correspond almost identically.

Encystment takes place precisely as in *Trichomastix*. Before encysting the animal develops a karyosome in its nucleus (fig. 25). The axostyle, undulating membrane and flagella are then gradually lost as the cyst is formed (figs. 26, 27). Finally the nucleus becomes drawn out in the cyst, and it is almost an impossibility to tell whether any given

cyst belongs to *Trichomonas* or *Trichomastix*, so closely do they resemble one another (cf. figs. 28, 15). Only by seeing the living animals encyst can one make quite certain.

Here, as in *Trichomastix*, there are no signs of any sexual process, either heterogamic or autogamic (see p. 227).

The account I have given of the life-history of the trichomonads is so different from those of others that I must here say a few words regarding certain points.

First as regards division. This has been said to occur by several observers, but details of the process are most meagre. For instance, Seligo (44) merely mentions the fact that he saw longitudinal division in *Trichomonas batrachorum*. In *Trichomonas lacertæ* Prowazek (73) believed that longitudinal division resembling that of *Trichomastix lacertæ* took place, but he was unable to find all the stages and his figure is unconvincing. He further described a multiple division, which, from what I have seen in *Trichomastix serpentis* (56), I believe to have been really a degeneration phenomenon.

Kunstler (63) stated that *Trichomonas intestinalis* (from the guinea-pig) divided longitudinally, and remained active during the process. But he gave no accurate details.

A description of the division of *Trichomonas intestinalis* from the mouse has recently been published by Wenyon (87). According to him, "there is a division of nucleus, blepharoplast, and of the peculiar pointed organ which projects from the posterior end of the animal. The undulating membrane and its support with the flagellæ (sic) appear to be new formations." Later he states that the "pointed organ"—i. e. the axostyle—"divides by longitudinal division and is the last part of the animal to divide." If Wenyon's description be correct, then the trichomonads of the mouse divide in a manner which is totally different from that of the forms which I have investigated. It appears to me probable, however, that Wenyon is mistaken, and that the appearances he has seen and

figured have been wrongly interpreted. His figures, 13, 15, and 21 (Pl. 11) are really drawn from dividing animals, I believe. They correspond closely with what I have myself seen. But the remaining figures of "stages in division" are, I think, nothing more than degenerate or fused monads. I have seen many similar appearances and feel convinced that they have nothing at all to do with division. It is remarkable also that no figure is given in which a splitting of the axostyle is shown. On the contrary, figs. 15 and 21 show a single rod extending from nucleus to nucleus—an appearance scarcely explicable on the assumption that the rod is formed from the split halves of the former axial organ.

In the second place it is to be noted that the cysts which I have found are quite different from those described in allied organisms. For the most part the accounts given (Kunstler, Perroncito, etc.) are too indefinite to allow of comparison being made, but in one case at least (Prowazek [73]) there exists a full account of encystment, and it differs widely from what I have seen. But it is fruitless to discuss the matter further at present.

I have never found any signs of the formation of those curious rounded-off, half-encysted forms, which, according to Wenyon, occur in *Trichomonas* from the mouse, and which probably bring about infection. I do not believe any such condition occurs in the trichomonads from frogs and toads.

Finally, it is necessary to say something about the problem of hosts and species. All I wish to say is that the trichomonads I have observed appear to me to be sufficiently well marked to be kept specifically distinct for the present. As is well known, a *Trichomonas intestinalis* has been described from many different hosts. Whether there is really one species, or more than one, our present state of knowledge does not permit us to decide. Similarly, in spite of their great resemblance, I believe *Trichomonas* and *Trichomastix* are sufficiently well distinguished from one another to be placed conveniently in different genera, without entering

into the endless discussion of "What is a genus?" or "What is a species?" It seems to me profitless to argue the matter further at the present time.

(c) Discussion of some Special Points in the Morphology and Life-history of the Trichomonads.

I.

The Blepharoplast.—I wish here to say something about the minute chromatic¹ body—the blepharoplast—which lies at the base of the flagella, and whose remarkable rôle in division I have already shown.

The name "blepharoplast" was introduced by Webber (86) for the small body, which lies beside the nucleus and gives rise to the cilia in the antherozoids of plants (cycads, ferns, etc.). It was previously described by Belajeff, to whose beautiful work (48, 49, 50) we owe much of our knowledge of its nature. Additional facts have been given by Ikeno (60), Shaw (80) and others. Although the earlier work was inconclusive, it seems practically certain, from the more recent studies—especially of Shaw and Belajeff—that the blepharoplast of the spermatozoid is really the centrosome or its derivative.

Amongst animals an exactly comparable condition—as I believe—is found. Here the axial filament of the spermatozoon tail arises, at least in many cases, from the centrosome—just as the cilia of the spermatozoid arise from the blepharoplast. This was first described by Moore (69), and has since been confirmed by a host of other workers (Hermann, Lenhossék, Meves, and many more).

Latterly the term "blepharoplast" came to be used to designate the chromatin body which lies at the base of the

¹ It can be stained not only by the iron-hæmatoxylin method, but also with Delafield's hæmatoxylin and borax carmine (not always satisfactorily with the last).

flagellar apparatus in trypanosomes—without, however, its strict homology with the organ in plant spermatozooids being insisted upon. Many different opinions have existed regarding the real nature of this body. Rabinowitsch and Kempner (75) regarded it as a “nucleolus,” though why it is difficult to see. Wasielewsky and Senn (85) believed it to be merely a cytoplasmic thickening—a structure independent of the nucleus. Laveran and Mesnil (65) considered that the blepharoplast should be regarded as a kind of centrosome—a view which they had already advocated in 1900 (‘CR. Soc. Biol.’). The view was based upon analogy with the structure of sperms and some flagellates, for no evidence of the blepharoplast functioning as a division centre had been brought forward. But it was a suggestive working hypothesis. Bradford and Plimmer (52) called the blepharoplast a “micronucleus,” because they believed it played a part in the “conjugation” which they observed. This comparison with the organella of Infusoria probably rested upon an incorrect interpretation of the phenomena observed.

The whole matter was apparently cleared up by Schaudinn (79) in his study of the life-cycle of *Hæmoproteus* (*Trypanosoma*) *noctuæ*. From this famous investigation it appeared that the trypanosome blepharoplast should really be regarded as a nucleus specially differentiated to subserve the locomotory functions of the cell—a kinetonucleus, which played its part in conjugation, etc., just like any other nucleus. Far from being itself a centrosome, Schaudinn showed that it actually contained a division centre, just like that of the trophonucleus.

Starting out from Schaudinn’s discoveries, Gross (59) made a very suggestive comparison between a sperm and a trypanosome, but in a converse manner to that which had been made by Laveran and Mesnil: that is to say, he suggested that the end-knob (centrosome) of the sperm might be regarded as a kinetonucleus, the sperm thus being binucleate like a trypanosome.

More recently a very careful investigation of the morpho-

logy of trypanosomes (*T. gambiense*, etc.) has been made by Salvin-Moore and Breinl (70). Their results are worthy of special attention, because their methods were greatly superior to those used by the majority of trypanosome describers. So convinced are these two investigators of the centrosome nature of the blepharoplast that they call it throughout "the extra-nuclear centrosome." They give excellent figures of its origin from the "centrosome," which originally lies in the middle of the synkaryon. I must point out, however, that although the blepharoplast here appears to be the sister of the "intra-nuclear centrosome"—which seems to act as a division centre in the "amitosis" these authors describe—there is, nevertheless, no proof that it possesses the most characteristic powers of a centrosome, that is, in bringing about nuclear division.

The first observers to describe the trypanosome blepharoplast as playing the part of a division centre are França and Athias (57), who have lately figured some remarkable stages in *T. rotatorium*. They describe irregular, amœboid stages which undergo "segmentation," during which the flagellum is lost, and the blepharoplast appears to divide and function as a centrosome during the nuclear divisions. Although this fits in so well with the views which I hold, I must confess that these forms, both from the description of their origin and from the figures, seem to me to be abnormal or degenerate.

Hartmann and Prowazek (25) have sought to bring the blepharoplast of *Trichomonas* and *Trichomastix* into line with their expanded version of Schaudinn's "Doppelkernigkeit" hypothesis. They base their views upon Prowazek's description (73) of the forms from the lizard. The karyosome is regarded by them as the kinetonucleus, boxed up in the trophonucleus. Hence they say that "the basal bodies¹ at the root of the flagella can be interpreted as daughter-centrioles of the karyosome-nucleus, and hence correspond with the derivatives of the centrosome in the tail

¹ What I call the blepharoplast.

filaments of spermatozoa." My own interpretation, that the trichomonad blepharoplast and the end-knob (not in any way the karyosome, however) are homologous, seems to me to fit in with the facts much more satisfactorily.

And this brings me to the point to which all the foregoing remarks are converging. In short, I believe that the divers structures with which we have been dealing—blepharoplast of fern, trypanosome and trichomonad, end-knob of sperm, and hence also centrosome—are all strictly homologous structures. That they are identical one cannot, of course, say. For the trypanosome blepharoplast is not, except in a very wide sense, a cytocentre. But functionally they are identical; in each they give rise to the locomotor organs—tail filament, undulating membrane or flagella, cilia—of the cell to which they belong. And from their behaviour one would suppose that they not only give rise to these organs, but also remain to preside over their functioning after their formation.

I do not wish to enter into a full discussion of this difficult matter here, so I will content myself with a very few further remarks. To do justice to the subject one would have to review a greater mass of literature than there is room for in this paper—all the work, that is, dealing with the so-called Lenhossék-Henneguy hypothesis.

When I mention the end-knob of the sperm and the blepharoplast of a fern or trypanosome as homologous, I do not mean to imply that similar organs in other organisms are not to be included in the same category. Indeed, I believe there are many other quite similar arrangements. It will suffice to recall the condition described by Ischikawa (61) in the spores of *Noctiluca*. The centrosome is here seen to lie at the base of the flagellum, just like a blepharoplast. It is difficult to believe that it could be other than homologous.

Further, the conditions described by Schaudinn in *Hæmoproteus noctuæ* are not necessarily antagonistic to this hypothesis. Assuming that these unconfirmed observations of Schaudinn are correct, it does not follow that they apply

equally to ordinary trypanosomes. Indeed, the careful work of Salvin-Moore and Breinl on a true *Trypanosoma* show that quite a different arrangement exists here. The blepharoplast in *T. gambiense* does not appear to be a nucleus containing a division centre like that in *H. noctuæ*. It is quite possible that in forms like *H. noctuæ* the "blepharoplast" is really a specialised nucleus¹ which is in connection with the real blepharoplast. There are many cases known, moreover, in which one solitary nucleus gives rise to the flagella (cf. Plenge [72], etc.).

It is difficult to believe, from their structure, that the blepharoplast of *Trypanosoma* and that of *Trichomonas* (cf. fig. 16, Pl. 2) are not homologous. And obviously the blepharoplast of *Trichomastix* is homologous with that of *Trichomonas* (cf. figs. 1 and 16). But then, again, it appears more than probable that the blepharoplast of the trichomonads is homologous with a centrosome (cf. figs. 6, 21—especially the latter). When the case of sperms is considered in addition, the homology appears to me almost established.

I do not for a moment suppose that either of these structures—blepharoplast and centrosome—is derived necessarily from the other. They are, according to my view, merely homologous organs—both originally, in all probability, derived from the nucleus. Their morphological similarity depends upon their physiological identity. Their nuclear derivation is seen, in many cases, in their staining reactions.

These points seem to me to be very clearly brought out in

¹ Since writing the above remarks, I have been pleased to find that my view fits in exceedingly well with the observations of Minchin (68). I think his view really corresponds exactly with mine, namely, that we may have, in connection with the locomotory organs, a specialised nuclear apparatus which is really to be regarded as kinetonucleus + blepharoplast. Minchin agrees with Keysselitz and others in word only—not in idea. For him there are two structures at the base of the locomotor apparatus—a kinetonucleus and a blepharoplast of a centrosomic nature. Of course, it does not in the least follow that all trypanosomes are built on the same plan as those in tsetse-flies.

the study of the division of the trichomonads recorded in preceding pages. To conclude my remarks, I will now sum up the views which I have attempted to express as briefly as possible in the foregoing pages, in the following words: The blepharoplast of the antherozoid, the blepharoplast of the trypanosome and trichomonad, and the end-knob of the axial filament of the metazoan sperm are all homologous structures, whose function is to provide for the locomotory activities of the cell. They are further homologous with—in some cases (e. g. in sperms) directly derived from—the centrosome of the resting cell.

II.

The Axostyle.—This organ may suitably be considered here, as it is very closely connected with the blepharoplast.

Regarding the function of this organella in the adult individual there is some diversity of opinion. I believe that its real function is entirely skeletal. It is merely an axial support.

From Prowazek's work (73) it would appear to be a kind of division-centre in addition. But, as I have already said, I believe this conception rests upon an incorrect interpretation of the appearances observed. Nor can I agree with Wenyon (87) that the axostyle is an organ for attachment. One has only to observe the living animal to see that it is never used for this purpose.

What I am particularly concerned with here is the origin of the axostyle. As I have already shown, it is absorbed before division and reformed by the division of the blepharoplast. If the blepharoplast itself is the homologue of the centrosome, then the homology of the axostyle with the central spindle¹ at once suggests itself. The homology is

¹ I use the term in its original sense (Hermann, 'Arch. mikr. Anat.', 1891), that is, for the spindle uniting the centrosomes, and around which the mantle-fibres of the achromatic spindle are arranged.

obvious, if we consider a stage, such as that shown in fig. 21, Pl. 2. The daughter blepharoplasts (centrosomes) lie at either end, united by the axostyle in its early stage of development. It clearly corresponds to a central spindle. Around it lie the mantle-fibres—never very strongly developed—and the chromatin, though never strictly divided into chromosomes, in the equatorial plate stage. Later stages in the development of the axostyle are fundamentally but stages of growth (e.g. fig. 9, 10, etc.).

It appears to me justifiable, therefore, to say that the axostyle is the homologue of the central spindle, each being a centrodemus.

An almost similar conclusion has been arrived at by Hartmann and Prowazek (25), though in a different manner, and, as I believe, from incorrect premisses. The forms considered were the trichomonads from the lizard, following Prowazek's description. They say that the axostyle is formed by the "Caryosom des Amphinucleus," but I can find no foundation for this statement. And further, "Die vermutlich mit dem Centriol in Zusammenhang stehende Rippe (Achsenstab) ist eine Art von Centralspindel und geht in die Rippe des Tochtertieres über:" which is in complete agreement with what I have just inferred from my own observations.

Some other interesting comparisons may be adduced in favour of this view. Compare, for example, the origin of the tail filament—also a supporting structure—in spermatozoa,¹ by an exactly comparable centrodermosis, as described by Gross (59). And this also corresponds with the origin of the flagellum and membrane in trypanosomes and allied forms. And further, compare this with the origin of the flagella from the central spindle in the spores of *Noctiluca* as shown by Ischikawa (61).

In the remarkably complicated flagellate *Joenia*, Grassi found an organ which seems to be an axostyle. The division of this organism has been investigated by Grassi and Foà

¹ Of *Pyrrhocoris*. This method does not seem to obtain in most other sperms.

(58), and furnishes some interesting details. Before division the axostyle ("mestolo") is absorbed. Then a spindle ("fuso") of unknown origin makes its appearance beside the nucleus. It elongates enormously and comes to lie between the daughter nuclei; and subsequently a portion of it at least takes part in the formation of the axostyle in the two daughter individuals. It seems quite probable that a condition identical with that seen in the trichomonads really exists here, but that it was not fully made out owing to the great complexity of structure in *Joenia*. At all events, the comparison is very suggestive.

Another interesting comparison may be made between the axostyle and the axopodial rays of the Heliozoa. *Camp-tonema* furnishes an excellent example of the connection between axopodium and nucleus (Schaudinn [77]) and well illustrates a condition analogous to that of nucleus and skeleton in trichomonads.

III.

Conjugation.—From what has already been written regarding the life-cycle of the trichomonads from frogs, it will be apparent that I am quite unable to bring forward any evidence regarding their sexuality. At no time have I ever found the slightest indication of the existence of any form of conjugation.

It was stated by Schaudinn (43) that a conjugation (heterogamic) takes place in the *Trichomonas intestinalis* in man. This has never been properly confirmed. Shortly after, Prowazek (73) described an autogamy in *Trichomastix* and a heterogamy in *Trichomonas intestinalis* from the rat. Peculiar structures, said to be stages in conjugation (autogamy) in *T. intestinalis* in man, have since been described by Ucke (84) and Bohne and Prowazek (51). And it is to these very questionable bodies that I presume Prowazek refers (74) as autogamic stages. Personally I cannot agree with this interpretation of the structures. I have good reason for regarding them in a very different light.

And hence, for my own part, I regard the conjugation of *Trichomonas* and *Trichomastix* as still undemonstrated.

Negative evidence is, of course, always inconclusive. The fact that I have never found any conjugation in trichomonads after observing many, many thousands, proves nothing—save that the process, if it occurs, is very uncommon and difficult to find. But the difficulties I have met in the course of my researches have also shown me time after time the caution which is necessary in investigations of this sort. It is not justifiable from finding flagellates and cysts, or things like cysts, together in the gut contents, to connect the two without further evidence. As I have found only too often, it is necessary to study all the organisms which occur in the gut; and not only the organisms but also all cell-remains and other débris. Only by conscientious adherence to this slow and tedious method can satisfactory results be obtained. It is a pity that this elementary and obvious precaution has been so frequently neglected.

(2) The Octoflagellate (*Octomitus dujardini* nom. nov.).

Although this minute organism is the commonest of all the flagellates which are found in the large intestine¹ of frogs and toads, nevertheless it is the one which has given me the greatest trouble; and about its life-history I have been able to discover but little. On account of its very small size and very complicated structure it is not surprising to find that it has never been accurately described. None the less it has been named a great many times, with the result that the literature and the available facts relating to it are at present in a hopelessly chaotic condition.

I will therefore first endeavour to summarise briefly the

¹ Since writing this account of the parasite it has been pointed out to me by Prof. Minchin that Danilewsky ('Parasitologie Comparée du Sang II,' 1899) observed the organism in the blood and body-cavity of sickly frogs, etc. This must be regarded most certainly, I think, as a pathological condition.

history of the animal. I have found it impossible to name without an exhaustive inquiry into all the available literature bearing upon it.

Only one serious attempt, that of Foà, has been made recently to classify this animal correctly, and her solution of the matter cannot be regarded as correct. At the time when I published my preliminary account I was unable to enter fully into a discussion of the matter. I therefore named the organism *Octomitus* sp., and I will now give my reasons for having done so.

The first authentic record of flagellates in frogs, so far as I have been able to discover, is that of Ehrenberg, 1838.¹ Ehrenberg distinguished two different organisms: *Bodo intestinalis*, and *Bodo ranarum*. The former he states to be $\frac{1}{7\frac{1}{2}}$ mm. long, occurring in the large intestine of frogs, the latter $\frac{1}{60}$ mm. long, and found in frogs and toads. His description and figures are naturally very incomplete on many points (e.g. the number of flagella), but it appears to me highly probable that *B. intestinalis* Ehrbg. is really the 8-flagellate parasite, and *B. ranarum* Ehrbg. is *Trichomonas* or *Trichomastix*. I think it is certain that neither really belongs to the genus *Bodo* as at present constituted.

In 1841 Dujardin established the genus *Hexamita*. He described three species: *H. nodulosa* and *H. inflata*, from stagnant water, and *H. intestinalis*. Unfortunately only *H. nodulosa* is figured. It shows six very distinct flagella. *H. intestinalis* is stated to occur in the intestine and peritoneal cavity "des Batraciens et des Tritons." There appears to me to be but little doubt that this was really the 8-flagellate parasite—only six of whose eight flagella Dujardin was able to count with the apparatus at his disposal.

Diesing in 1850 describes, though apparently without any justification, the *Hexamita intestinalis* of Dujardin under the new name of *Bodo* (*Amphimonas*) *decipiens* Diesing. He makes no original observations on the organism.

¹ But they were possibly first observed by Leeuwenhoek in 1702.

Burnett, 1851, mentions the presence of "Bodo (Ehr.)" in the frog, and records a few observations on the organisms. But I am quite unable to decide which of the flagellates in the frog he really saw.

Perty was the first, in 1852, to distinguish *Trichomonas batrachorum* from the other flagellates. But he also appears to have recognised a flagellate which he calls *Cercomonas ranarum* (Bodo sp. of Ehrbg.). Probably this was the 8-flagellate once more, under another name.

Leidy, 1856, recognised both Ehrenberg's forms of Bodo, retaining the latter's name, *B. intestinalis*, for the smaller form.

The next change of name was brought about by Diesing, 1865. He describes *Hexamita intestinalis* Duj. as *Amphimonas intestinalis*. This name cannot be retained. The genus *Amphimonas* was made by Dujardin in 1841, and included three free-living species, each possessing two or three flagella. There is no justification for Diesing changing Dujardin's own genera in this way.

Stein's great work on flagellates appeared in 1878, and in it he describes, with tolerably accurate figures, a parasite said to be common in frogs, under the name *Hexamita intestinalis* Dujard. Although Stein only figures six flagella, I think there can be no doubt that he really saw the 8-flagellate organism. The rest of his description is fairly good.

Bütschli, in the same year (1878), resumed the investigation of the free-living forms. He states that there are really eight flagella in these organisms and unites Dujardin's two species, *Hexamita nodulosa* and *H. inflata*, into one species, *Hexamitus inflatus*, thus modifying the original name. It must remain doubtful whether Bütschli's 8-flagellate organisms were really the same as Dujardin's 6-flagellate animals.

Further complications were brought about by Grassi in 1879. He proposed the generic name *Dicercomonas* for two different parasitic flagellates. The genus was dis-

tinguished from his other genus *Monocercomonas* (*Trichomonas* of others) by the one character "a coda bifida." He divided the genus *Dicercomonas* into two sub-genera, *Monomorphus* and *Dimorphus*, of which the definition is, to say the least, scanty. *Monomorphus* is distinguished as "si presenta sotto una sol forma." The only species is *Dicercomonas* (*Monomorphus*) *ranarum*, with "*Hexamita ranarum*, Duj."¹ given as a synonym. The name *Dimorphus* was given to *D. muris*, and subsequently eliminated as *Megastoma entericum* (Grassi, 1881).

Saville Kent, 1880, re-described *Hexamita intestinalis* Duj. from his own observations. His account is in many ways inaccurate, and he persists in the statement that there are six flagella: "The exact number, character, and point of insertion may be readily substantiated . . ." I feel convinced that he really saw the 8-flagellate organism. He enumerates further both Ehrenberg's *Bodo* forms, but made no observations himself upon them. He suggests, however, that *Monas intestinalis* Dujardin is a synonym for *Bodo intestinalis*. This appears to me highly improbable.

Grassi re-described the organism under consideration in 1882. He was unable to determine the number of flagella, and apparently relinquished the name *Monomorphus*. For he adheres to the name *Dicercomonas intestinalis* Duj., giving *Hexamita intestinalis* Duj. as only synonym.

In the same year Kunstler (1882) described—though very briefly—a flagellated organism from tadpoles, which appears to me to have been probably our 8-flagellate organism. The flagella were not accurately investigated. Kunstler, in spite of his insufficient observations, introduced the new name *Giardia agilis* for this animal.

Bütschli, 1884, retains the genus *Hexamitus*² (Duj. emend. Büt.).

¹ A mistake for *Hexamita intestinalis* Duj.

² Giving *Chætomonas* Ehrbg. and *Heteromita pusilla* Perty as possible synonyms for *Hexamitus*. Neither of these appears to me to have anything to do with the form under consideration.

In 1885 Seligo again described a 6-flagellate parasite from various frogs, etc., employing the name *Hexamitus intestinalis* Duj. for it.

Grassi, 1888, maintained his former genus *Dicercomonas*, but gave a better definition. He, however, recognised only "quattro flagelli anteriori." He gave as synonyms *Hexamita* Duj. and *Giardia* Kunstler. For the free-living forms he proposed to replace the name *Hexamita* Duj. by the new name *Dujardinia* Grassi, which, if adopted, would thus abolish the name *Hexamita* entirely.

Klebs, 1892, retained Bütschli's nomenclature (*Hexamitus intestinalis*, Duj.), though recognising for the first time that this flagellate really possessed "stets sechs vordere und zwei hintere Geisseln, so dass die Gattung eigentlich *Octomitus* heissen müsste." And he adds, "Doch erscheint es passender, den alten eingebürgerten Namen zu bewahren."

Senn, in 'Engler and Prantl,' 1900, also retains the name *Hexamitus intestinalis* Duj., and gives as synonyms for *Hexamitus*, *Heteromita pusilla* Perty, *Amphimonas* Diesing, and *Dicercomonas* Grassi—evidently copied from Bütschli. Four pairs of flagella are described.

Doflein, 1901, again attributes but six flagella to this animal, and retains Bütschli's name.

Stiles, 1902, made an attempt to arrive at a definite understanding regarding the nomenclature of this and other flagellates, but his work was entirely of a literary nature, and not based upon any further investigation of the organisms themselves.

Moroff, in 1903, was responsible for yet another change in the name of this parasite. He observed a similar organism in a fish, but stated (though his figures and description do not bear this out) that it was the same as that found in Amphibia, and there known as *Hexamitus intestinalis* Duj. He proposed to change the name, however, to *Urophagus intestinalis* (Duj.) Moroff,¹ because of the presence

¹ Wrongly giving *Hexamitus intestinalis* Dujardin, 1841, as synonym.

of eight flagella. How absolutely unwarranted such a change is will easily be seen when it is recollected that the genus *Urophagus* was founded by Klebs himself, the first to observe eight flagella in the parasitic form. And Klebs founded this genus to contain a single species, which differed from all the other 8-flagellate organisms in the fact that it ingested food at the hind end of the body—an act which Moroff never observed.

The first real attempt to describe the structure of this animal was made by Foà, 1904, who also made an attempt to assign the correct name to the organism. She says: "Grassi (1888) conferma la propria classificazione," and accordingly names the parasite *Dicercomonas intestinalis* (Duj.).

Now I must point out that this name adopted by Grassi and Foà is not available. The genus *Dicercomonas* was founded by Diesing in 1865,¹ and not by Grassi in 1879. Diesing's definition runs as follows: "*Dicercomonas* Diesing (monadis spec. Perty). Animalcula solitaria libera symmetrica. Corpus immutabile, ovale, hyalinum, caudiculis duabus retractilibus, nec ciliatum, nec loricatum. Os terminale. Flagellum unum pone os. Anus . . . Ocellus nullus. Partitio . . . Anodontarum parasita." He then enumerates a single species, *Dicercomonas succisa* Diesing (syn. *Monas succisa* Perty) found "in aqua cum Anodontis putrescentibus." It thus appears that Grassi's name must be relinquished, although it is just conceivable that Diesing really observed a similar organism, for Certes (1882) described *Hexamita inflata* Duj. as occurring in the oyster. However, we must take Diesing's definition as it stands—as that of a uniflagellate.

Finally, Kunstler and Gineste, in 1907, described another species of "*Giardia*," namely *Giardia alata* K. et G. from tadpoles of a frog. From their description this hardly seems to agree with my observations on the structure of the parasite under consideration. On the contrary, the new form appears more closely allied to *Lamblia*. However, it is just

¹ Not 1856, as given by Stiles.

possible that it is really the common form from the frog, and hence this must be considered as a conceivable synonym.

Now if we agree, as is usual, to take *Hexamitus inflatus* as the type species—a form with six flagella, and of free-living habit, as described by Dujardin—we are left without any generic name to bestow upon our parasitic form. Bodo Ehrenberg is unavailable; so also *Amphimonas* Dujardin and *Dicercomonas* Diesing. *Cercomonas* and *Urophagus* are quite distinct genera, and *Giardia* is too inadequately described to be adopted with certainty. That is why I proposed (12) to employ the name *Octomitus*, as originally suggested by Klebs. This name seems to me to be the most suitable for this and similar forms. But if we agree to call the animal by this name another difficulty at once presents itself. The genus *Octomitus* was created by Prowazek¹ in 1904 to include a single species, *O. intestinalis* from the rat. But this is the very name—*Octomitus intestinalis* Duj.—which our parasite would have to receive. Hence we arrive at another obstacle. Now it is quite probable that Prowazek's organism is only a form of the animal usually described as *Hexamitus* or *Dicercomonas muris* Grassi. Wenyon, however, thinks that there are probably two different species included under this title, in which case it would be best to let Prowazek's name stand.

I propose, therefore, to create the new specific name *dujardini* for the octoflagellate parasite of frogs and toads, whilst referring it to the genus *Octomitus*. This, I believe, will effectually surmount all difficulties, and will also take cognisance of the probable discoverer of the animal.

The genus *Octomitus* will, therefore, contain three² species of parasitic flagellates, namely:

1. *Octomitus dujardini*, in frogs and toads.
2. *Octomitus muris* Grassi, in rats and mice (the narrow form of "*Hexamitus* [*Dicercomonas*]" *muris*).

¹ Though written by him "*Oktomitus*," and without any indication that the name was being employed for the first time.

² The forms in tortoises, fish, oysters, etc., are too little known to warrant the giving of specific names to them at present.

3. *Octomitus intestinalis* Prowazek, also in rats (the broad form).

Hence I can now sum up the nomenclature, and will then proceed to a consideration of the organism itself. The name stands as follows :

OCTOMITUS DUJARDINI nom. nov.

Syn.: ? *Bodo intestinalis* Ehrenberg, 1838.

Hexamita intestinalis Dujardin, 1841.

? *Bodo* (*Amphimonas*) *decipiens* Diesing, 1850.

? *Bodo* (Ehrbg.) Burnett, 1851.

? *Cercomonas ranarum* Perty, 1852.

? *Bodo intestinalis* (Ehrbg.) Leidy, 1856.

Amphimonas intestinalis Diesing, 1865.

Hexamita intestinalis (Duj.) Stein, 1878.

Dicercomonas (*Monomorphus*) *ranarum*
Grassi, 1879.

Hexamita ranarum (Duj.) Grassi, 1879.

Hexamita intestinalis (Duj.) Kent, 1880.

? *Bodo intestinalis* (Ehrbg.) Kent, 1880.

Dicercomonas intestinalis (Duj.) Grassi, 1882.

? *Giardia agilis* Kunstler, 1882.

Hexamitus intestinalis (Duj.) Bütschli, 1884.

Hexamitus intestinalis (Duj.) Seligo, 1885.

Dicercomonas intestinalis (Duj.) Grassi, 1888.

Hexamitus intestinalis (Duj.) Klebs, 1892.

Hexamitus intestinalis (Duj.) Senn, 1900.

Hexamitus intestinalis (Duj.) Doflein, 1901.

Hexamita intestinalis (Duj.) Stiles, 1902.

Urophagus intestinalis (Duj.) Moroff, 1903.

Dicercomonas intestinalis (Duj.) Foà, 1904.

? *Giardia alata* Kunstler et Gineste, 1907.

Octomitus sp. Dobell, 1908.

Structure.—The general shape of *Octomitus dujardini* is fusiform or elongate oval (see Pl. 3, figs. 29, 31). An average adult individual measures about 10 μ in length. The nucleus and the organellæ connected with it present a consider-

able degree of complexity. The nucleus itself may be regarded as consisting of three pairs of structures. These all lie at the anterior end of the animal, and are arranged roughly in the shape of a horse-shoe. At the extreme anterior end are two minute granules of chromatin, lying side by side, connected with one another by a delicate filament of chromatin, or else in close apposition. Immediately behind this pair, and united to it, is another pair of chromatin granules. These are also connected with one another across the middle line. It will thus be seen that the two pairs of granules form the four corners of a minute square area, free from chromatin (cf. fig. 29). The main part of the nucleus consists of a large lobe of chromatin on either side, connected with, and extending backwards from, the posterior pair of chromatin granules.

Extending backwards from the posterior pair of granules are two delicate rod-like structures, which I believe to be homologous with the axostyle of trichomonads. I shall therefore employ the same name to describe them. Each axostyle terminates at the extreme posterior end of the animal in a minute chromatic granule. The eight flagella arise as follows: From the anterior end six, from the posterior two. The anterior take origin from the two pairs of chromatin granules, one pair of flagella arising from the anterior, and a single flagellum arising from the posterior on either side (cf. fig. 29). The posterior flagella, or, as I shall call them, the caudal flagella, arise from the chromatin granules at the posterior extremities of the axostyles. The length of the flagella is variable, but is frequently great. I have not unfrequently found individuals in which the caudal flagella had attained a length of over 30μ , or more than three times that of the body. In consequence of their length and the minute dimensions of the animal there is often great difficulty in counting these appendages.

The axostyles are normally parallel, but they frequently get crossed, owing to the twisting movements of the animal (see fig. 30). This crossed condition, therefore, cannot be regarded as the normal condition, though the *Octomit* in the rat

has been usually so described. Anyone who will take the trouble to watch an *Octomitus* continuously for several hours can convince himself of this. When the animal is moving quietly and has ceased to dart about, the axostyles invariably appear parallel with one another (see fig. 31). This crossing of the rods during active screw-like movements, moreover, negatives the suggestion of Prowazek that these structures, in *O. intestinalis*, are really not rods, but the sides of a tube seen in optical section. The axostyles have also been frequently interpreted as continuations of the caudal flagella (cf. Foà, etc.)

I have described the nuclear apparatus as it appears to me most usually to exist.¹ But there are other variations often met with. It is very commonly found that the two anterior pairs of granules are fused or superimposed, so that they cannot be made out clearly (cf. fig. 30). Many considerations have led me to believe, however, that the nuclear chromatin is really arranged in the three pairs of parts which I have described. A very striking confirmation is seen in a degenerate form, which is not uncommon in old cultures (see fig. 37, Pl. 3). In this the nucleus has degenerated and broken up, but into three pairs of granules. These forms have died and cast off their flagella. The nucleus has been resolved, I believe, into its component parts.

Very many other degenerate forms have been encountered. I will here mention only one more, which is very striking in appearance. In this (see fig. 36) the nucleus has fragmented, and the fragments have run along the axostyles, so that they present the appearance of strings of beads.

There is no cytostome and no contractile vacuole.

Octomitus dujardini occurs in *Rana temporaria*, *R. esculenta*, and *Bufo vulgaris*, and is equally common in all of them. It occurs also in newts.

¹ It is worth noting the extraordinary way in which all the parts of the nucleus and its connections are paired, thus giving rise to a very well-marked bilateral symmetry. It is interesting, too, to compare this form with other similar forms, e. g. *Lambliia* (cf. Metzner [66]).

I must here say a few words about some of the descriptions which have previously been given of this animal.

Stein (46) described it as having six flagella and a spherical "kernähnliches Körperchen" at the anterior end. He also described a terminal mouth and a contractile vacuole, and figured forms "mit zwei seitlichen Reihen undulierender Fortsätze am Vorderleibe." The axostyles are recognisable in some of his figures.

Saville Kent (26) says there is a "spherical, subcentral endoplast," and the body is "frequently with one or two longitudinal dorsal sulci" (? the axostyles). A contractile vacuole is said to be present, and is figured at the anterior end. Seligo (44) also found a bladder-like nucleus with a nucleolus lying near the middle of the body.

Apparently the axostyles were first recognised by Grassi (23), for he describes the organism as possessing a "scheletro interno (fatto da uno o due pezzi?)" The axostyles were indicated by Klebs also (27), when he described the body as being furnished "mit zwei schraubig verlaufenden seichten Längsfurchen, von denen je ein Rand stärker als Längskante vorspringt." No contractile vacuole was observed by him, and the nucleus was stated to be anteriorly situate.

The most accurate account yet given is that of Signa. Foà (18). She describes the anterior flagella as arising, three on either side, from a pair of "blepharoplasts," and interprets the main lateral lobes of the nucleus as "karyosomes." And she also saw a figured two longitudinal lines (the axostyles) running down the body. Her account is evidently based on careful observations.

The body so often described in the middle of the animal as the nucleus was probably a food mass. Such masses are often present, though how they get there I do not know, as there is no mouth. During degeneration, large vacuoles usually appear in the protoplasm, a large one often making its appearance immediately behind the nucleus. It is these vacuoles—which are not normally present—which have probably been taken for contractile vesicles. The frequently

seen bifid condition of the caudal extremity is also not a constant feature. It owes its formation to the rigidity of the skeletal rods and the mobility of the cytoplasm in which they are imbedded.

I have never seen anything corresponding with the undulating processes described by Stein.

So far I have described the structure of adult individuals only. But in addition to these there are usually to be found a certain number of small forms. Many of these are exceedingly minute—not reaching a greater length than $2-3\mu$ —and are of a simpler structure than the fully grown animals. Even in the smallest forms, however, when it is possible to make an accurate count of the flagella, there are always eight present. But some of the tiniest organisms appear to have only one axostyle (see fig. 32, Pl. 3). Stein has figured the young form with four flagella and one axostyle.

The shape of the smallest individuals is more rounded than that of adults. It is the nuclear apparatus, however, which shows the greatest differences. In the smallest forms (see fig. 32) the nucleus consists of a few loosely packed chromatin granules, and all the anterior flagella appear to be rooted in it. At other times the nucleus has a distinct karyosomic granule, and the flagella arise from minute basal granules on the periphery (see fig. 33). Later stages show a gradual transition to the bilobed nucleus of the adult (fig. 34), and many very small animals appear—as far as details can be made out—to be identical with adult individuals (fig. 35). The origin of these small forms is still unknown to me.

Movements.—When freshly removed from their host these animals display a remarkable degree of activity. They move at such a pace that it is quite impossible to make out anything of their structure as they dart across the field of the microscope—a mere dot of protoplasm surrounded by a blur of flagella. After a short time, however, they slow down, and one is able to watch their movements with ease.

In a slowly moving animal all the details of structure—save the most minute points in connection with the nuclear apparatus—can be made out, with patience, with almost as much certainty as in a stained specimen.

The body is characterised by extreme flexibility, which enables the animal to double and twist itself in all directions. Movement always occurs in a forward direction—that is, with the nuclear end in advance.

During progression it is only the anterior flagella which are lashed about. The caudal pair are usually trailed. Not uncommonly they become attached to some object, and thus serve to anchor the organism, which may then rotate about the fixed point. Saville Kent and others have already noticed this.

Multiplication.—In spite of having examined countless thousands of individuals, both alive and in fixed and stained preparations, I am still uncertain of the method of reproduction. I have many times found stained specimens which are identical with those described as division-stages by Foà and Wenyon in the *Octomitus* in rats. But from observations on the living animals I am now satisfied that these stages are merely degenerate and fused forms, which have nothing whatever to do with division. Bütschli states that "Theilung" occurs in *Hexamitus inflatus*, but beyond figuring an animal with two spherical nuclei and four caudal flagella he gives no details of the process. According to Prowazek, in *Octomitus intestinalis* "bei der Teilung scheint die Achsenröhre¹ ganz nach Art des Achsenstabes der Trichomonaden und -mastiginen zu funktionieren. Sie nimmt eine etwas spindelförmige Gestalt an, die vornehmlich durch eine Anschwellung des äusseren Belages hervorgerufen wird." Nothing further is said or pictured of the division.

I have on several occasions found stained specimens which appear to me to represent genuine stages in division. These are unfortunately extremely rare, and have all been at approxi-

¹ The axostyles were thus interpreted.

mately the same stage. It is therefore impossible to describe the whole of the process.

Two of these stages are figured in Pl. 3 (figs. 40, 41). According to my interpretation it appears probable that division is longitudinal and effected in the same way as in the trichomonads—allowing, of course, for the more complex structure. Before division the axostyles would therefore be absorbed, and with them the caudal flagella. A stage with only six flagella—all at the anterior end—would thus result. We do, indeed, find such organisms on rare occasions (see fig. 46), but I am inclined to think that they belong to a different species (see p. 245). However, they possibly belong here. The nucleus would subsequently divide, new flagella would make their appearance, three at either end, and we should expect to see two axostyles lying between the nuclei as they separate. This is the condition which I imagine is seen in figs. 40 and 41. Later, when the axostyles had elongated and the animal had been constricted into two, the caudal flagella would make their appearance, either by a new growth or by the drawing out of the axostyles at the point of severance. Both the organisms figured were very distinct, and in fig. 41 the suggestion of the outgrowth of new flagella, as in *Trichomastix*, is very strong. The bipartite nuclei are also very striking, and it seems difficult to regard these forms otherwise than as division stages. But as I have already indicated, the evidence of division is by no means conclusive. I give these few observations because I have completely failed to discover anything more, and because the descriptions of division in similar forms seem to me to be incorrect.

Encystment.—When the organisms are artificially removed from their host or liberated in the fæces they nearly always die. For a long time I was unable to discover the cysts or the method of dissemination in nature. On a few occasions, however, I have found the permanent cysts of *Octomitus*, though they have never occurred in anything but very small numbers. The cysts are small and usually

oval (fig. 38, Pl. 3), are slightly yellowish, and contain a single individual. The axostyles are not as a rule very distinctly seen, and there are no flagella present.

On a single occasion I have seen a cyst containing a monad which had become motile, having eight flagella, inside its cyst (fig. 39). After moving about actively, stretching the cyst in all directions, the monad subsequently escaped and swam away.

As in the case of the trichomonads, I have absolutely no idea what the influence is which causes the animals to encyst. Temperature, nutrition, drying, etc., appear to take no part in bringing about encystment.

Regarding sexual phenomena, I can merely repeat that I have never seen any conclusive evidence that conjugation takes place. Prowazek has stated that conjugation (heterogamy) occurs in "*Hexamitus intestinalis*" from *Tes-tudo græca*, but I cannot regard it as proven. The conjugation is said to be similar to that of *Trichomonas*. It may be added that Wenyon's careful investigation of similar forms in the rat resulted in observations similar to mine—namely, the discovery of monozoic cysts without any trace of conjugation.

(3) *Monocercomonas bufonis* Dobell.

On two occasions I have encountered in the toad a quadri-flagellate parasite, which differs considerably from *Trichomastix*. Although rare, the organism was present in great numbers in the infected animals. These, it may be noted, were both captured in the same place.

I have referred the animal to the genus *Monocercomonas* Grassi, because I believe the genus *Tetramitus*, to which similar organisms belong, ought to be reserved for free-living forms. And although the genus *Monocercomonas* is not very well defined,¹ it has already been used for

¹ The type-species is probably *Monocercomonas melolonthæ*, but Grassi's descriptions and figures are not easy to deal with. He has variously given *Trichomonas* (1879) and *Trichomastix* (1888) as synonyms for *Monocercomonas*.

parasitic flagellate forms with four equal anterior flagella. I think it best to retain this genus, therefore, for the parasitic quadriflagellates—reserving *Tetramitus* for free forms.

The general structure of the animals is shown in figs. 49 and 50, Pl. 3. Two different forms are here seen—a small, slender *Crithidia*-like form (fig. 49) and a larger and broader one (fig. 50). The size of the small forms is about $10\ \mu$ – $12\ \mu \times 2\ \mu$. The larger forms reach dimensions up to $20\ \mu \times 7\ \mu$. All intermediate sizes occur.

One of the features which most markedly distinguish this organism from those already described is the presence of a very well-marked cuticle. This is best seen, perhaps, in Giemsa preparations, where it stains pink, in contrast with the blue cytoplasm.

The four flagella are equal in length, and are all directed anteriorly: that is to say, there is no “*Schleppgeissel*” as in *Trichomastix*.

The nucleus is a large, oval body, composed of loosely-packed chromatin granules. It is placed anteriorly. The origin of the flagella is immediately in front of the nucleus. Sometimes they appear to arise directly from it (fig. 49), whereas at other times they seem attached to a small granule lying above and independent of the nucleus (fig. 50). Several vacuoles are usually seen in Giemsa preparations, but these are not visible in the living animal. There is no cytosome or axostyle.

When alive the organism progresses by characteristic jerky movements, rather like a *Bodo*. They are exceedingly active when first removed from their host.

Owing to the paucity of material I have not been able to ascertain anything of the life-history. In cultures of the toad's faeces all the animals died without showing any signs of encysting. From the fact that I have several times observed—in stained preparations—animals with eight flagella, it appears probable that they divide longitudinally in the usual flagellate manner. But the nuclear division and subsequent stages I have not been able to find.

(4) Notes on other Flagellate Organisms.

In the course of my work I have come across several doubtful organisms, which I will briefly describe here. They have been found, for the most part, in fæces cultures. I believe that they are in no way related to the other forms described in this paper, but that their presence was due to accidental inoculation of the cultures. However, their possible connection with other forms is not excluded, and I will therefore describe them. They are all of them uncommon.

1. A minute uniflagellate monad (Pl. 3, fig. 47). Length 3μ – 6μ . Sometimes shows a tendency to become amœboid. Stained specimens show a nucleus centrally situated, and consisting merely of a minute chromatin granule. Seen on several occasions in fæces of *Rana temporaria* and once in *Bufo vulgaris*.

2. Bodo sp. (Pl. 3, figs 42–45). Found on two occasions in fæces of *Bufo*.¹ Shows typical Bodo structure—two flagella, etc. (fig. 42). Length, up to 15μ . Hinder end often becomes amœboid, forming hyaline pseudopodia (fig. 43). Nucleus central. Very tiny forms sometimes seen (? another species), not measuring more than 3μ in length (fig. 44). On one occasion I found—in a preparation with free forms—a cyst which appeared to contain four Bodos and a residuum (fig. 45). As no flagella could be seen it is possible that the cyst belongs to some other animal. But it is interesting to record its presence, since Bodo may divide into four, after encysting, according to Prowazek. The length of the cyst was 21μ . I was unable to break it, owing to the presence of much sand in the fæces preventing the coverslip from being pressed against the slide. Though watched for several hours no movements of any sort took place.

3. A triflagellate monad (fig. 48, Pl. 3). Found only once,

¹ I have found a very similar Bodo parasitic in the large intestine of the common newt. It is remarkable for the possession of a very large blepharoplast-like body (Geisselsäckchen) at the base of the flagella.

in small numbers, in fæces of *Bufo*. The three flagella are separated at their origin, and equal in length. Length about $6\ \mu$. Movements sluggish.

4. An organism with six flagella. Several specimens found on different occasions in the fæces of *Bufo vulgaris* and *Rana temporaria*. Nucleus spherical, granular, anterior in position. Six equally long anteriorly directed flagella. No axostyles. Length about $10\ \mu$ (Pl. 3, fig. 46.) May possibly be a degenerate or developmental form of *Octomitus* (cf. p. 241), with which it was always found.

I may add that I have several times observed the *Bodo* described above undergo a process of degeneration which is remarkable for the formation of long, delicate, heliozoon-like pseudopodia. In this radiate condition the animal bears some resemblance to the multiciliate creature described in frogs by Grassi. According to Schuberg this is really a detached epithelium cell, but Grassi denies this. The name *Grassia ranarum* was given to it by Fisch. I regard its existence as highly doubtful.

B. RHIZOPODA.

(1) *Entamœba Ranarum* Grassi.

Syn.: "Amöbe" Lieberkühn, 1854.

Amœba ranarum n. sp., Grassi, 1879.

"*Amœba ranarum* (?) (mihi)" Grassi, 1882.

"Amöbe" Brass, 1885.

Amœba ranarum (Grassi) Doflein, 1901.

Entamœba ranæ Hartmann, 1907.

Entamœba ranarum (Grassi) Dobell, 1908.

The existence of an amœba in the intestine of the frog was first pointed out by Lieberkühn (1854), whose observations, as far as they went, were very accurate. He was able to distinguish it from leucocytes found in the same place, though it is not certain that the amœba which he saw and figured

was the form which I am about to describe. It is quite possible that he observed the amœboid stage of *Chlamydo-phrys*,¹ which I shall describe later. Lieberkühn did not name the organism, and as far as I am aware no name was given to it until 1879, when Grassi proposed the name *Amœba ranarum*. In the meantime, however, its existence had been recognised by Leuckart and others. Grassi's form is perhaps the same as mine, though this is not certain as no really accurate description of the animal has yet been given. Doflein (14) retained Grassi's name. I presume, moreover, that it is this form to which Hartmann (24) refers as *Entamœba ranæ*.

It was pointed out by Casagrandi and Barbagallo (54) that the parasitic amœbæ should probably be separated generically from the free-living *Amœba*. They proposed the new genus *Entamœba*, therefore, to contain the parasitic forms found in man and in the cockroach. There can be small doubt that this is justifiable. And the proposal was adopted by Schaudinn (43) in his work on the amœbæ in man.

Although the life-history of the amœba in frogs appears to differ considerably from that of other parasitic amœbæ,² I think it best at present to place it in the genus *Entamœba*. Assuming, then, that this organism is the same as that described by Grassi, it follows that its correct name is *Entamœba ranarum* Grassi.

Lieberkühn stated (37) that the organism occurred frequently in the large intestine of its host, sometimes being present in considerable numbers. He noted that it contained a number of granules, one of which (? the nucleus) was often of specially large size. Ingestion of food and division, though constantly sought, were never observed.

To this account Grassi (21) added the following facts.

¹ This also applies to the amœbæ described in frogs by other investigators—especially Grassi, whose description corresponds much more closely with *Chlamydo-phrys* than with *Entamœba*.

² And though *E. coli* and *E. muris* are very much alike, they appear to differ very greatly from *E. blattæ* as regards life-history.

The organism occurs in *Rana esculenta* captured in Rovellasca, Pavia, and Como. It is invariably present at all seasons and is sometimes very abundant. It was never found in toads. Regarding its structure, he says that an ectoplasm and endoplasm are distinguishable; that there is a round nucleus, with a nucleolus; that the form of the body is very variable, the protoplasm being almost liquid ("scorrevolissimo quasi fosse liquido"). Movement is rapid and effected by the thrusting out of digitiform pseudopodia, which may also be thrust in and out, however, without the animal changing its position. No contractile vacuole was noticed. The dimensions are stated to be as follows: Diameter, when rounded, from $8\ \mu$ to $24\ \mu$; when digitiform, up to $30.3\ \mu$ in length; diameter of nucleus never greater than $4.4\ \mu$.

Additional statements regarding the life-history have been made by Brass (2) and Hartmann (24). According to the former, the amœbæ are able to reproduce by division, by formation of swarm-spores and by means of resting spores. According to the latter, an autogamy similar to that of *Entamœba coli* occurs in *Entamœba ranarum*, though the observations upon which the statement rests are as yet unpublished.

I will now give my own observations on the animal. They differ in many respects from those of others.

Regarding the host, I can state that *Entamœba ranarum* occurs in *Rana temporaria* (Cambridge and Munich), *Rana esculenta* (Munich), and *Bufo vulgaris* (Cambridge). I have found it most frequently in *Rana temporaria*, about 23 per cent. of individuals examined being infected. Occasionally the parasite is present in immense numbers. It is also noticeable that the infection is local, for I often found that nearly all the frogs captured together in certain places were infected, whilst of others taken in a different area not one harboured the parasite.

Whether the animal exercises any injurious effect upon its host or not must remain an open question. Certain it is, however, that sometimes when the parasites are numerous

there are also present many blood-corpuscles and broken-up epithelium cells in the large intestine. These are readily ingested by the amœbæ. But their presence may be due, as Neresheimer (40) believes, to the injurious action of the intestinal worms which are always present in greater or less numbers.

All attempts to cultivate *Entamœba ranarum* on Musgrave and Clegg's medium have failed.

Structure.—This amœba is remarkable for the ease with which its structure at all stages of development can be seen during life. For instance, the nucleus can, with proper illumination, etc., be seen in the living animal just as plainly as in a fixed and stained specimen.

When an ordinary individual is examined in the living state it presents all the features usually seen in any amœba (see fig. 52, Pl. 4). There is no sharply-marked differentiation into ectoplasm and endoplasm; there is no contractile vacuole; there are the usual food bodies present more or less plentifully; but the most distinctive feature is the nucleus. By far the greater part of its chromatin is distributed at the periphery, so that in optical section the nucleus is always seen as a beaded ring (figs. 52, 53). Staining shows that a part of the chromatin is also distributed inside in the form of minute granules of varying size, arranged in a more or less distinct network (fig. 53). There is no caryosome (cf. Grassi). In fixed and stained animals the cytoplasm shows a very distinctly alveolar structure (cf. fig. 53, etc.).

It is, of course, very difficult to give exact measurements of an organism such as this. When more or less rounded the ordinary individuals measure about 20—30 μ in diameter. The nucleus is more easily measured. Its diameter is usually about 6 μ (cf. Grassi).

Very much larger organisms are sometimes to be found (fig. 58). They are often stuffed with food to a most surprising extent, but are nevertheless very active. The largest I have found measured over 60 μ in length when in a very slightly extended condition. In these forms the nucleus

becomes modified (fig. 59). It increases in size, reaching a diameter of 8-9 μ , and nearly all the chromatin passes to the periphery, so that the inside is quite pale in a stained preparation.

I have usually met with these large forms in cultures of the fæces. I believe they are to be regarded as abnormal animals, overgrown from over-feeding. Such hypertrophied organisms seem to be incapable of either dividing or encysting. They have always died when kept under observation.

In addition to the ordinary adult animal and the hypertrophied form, there are also to be found amœbæ of much smaller size (fig. 54). They are very much less common, but from the occurrence of all stages intermediate between them and the adults, I have no doubt that they are really the young forms. In addition to their small size they are characterised by possessing a different type of nucleus: for it is spherical, with a small but very distinct karyosomic granule (fig. 54). The diameter of the nucleus is 3-4 μ .

Although the animals must sometimes divide very rapidly—judging from their great abundance occasionally—it is extraordinarily difficult to find stages in division in preparations. I have never seen division in the living animal, though it is not for want of seeking for it. In preparations also I have encountered but few dividing animals, and these, unfortunately were all in approximately the same condition. Fig. 55 shows an organism with a very distinct dividing nucleus. From the occurrence of a binucleate stage (fig. 56), it is probable that fission of the cytoplasm does not take place till some time after that of the nucleus. It seems that the nuclear division is a kind of very primitive mitosis, similar to that seen in the cysts (see *infra*), where I have been able to follow it in considerable detail.

Occasionally one encounters forms like that depicted in fig. 57, in which an amitotic division of the nucleus is very strongly suggested. But observation of the living animal shows that such a state has absolutely nothing to do with division. The shape of the nucleus is constantly changing

with the movements of the animal, and with the change of position of the food masses lying in the cytoplasm. The condition figured is brought about by the pressure upon the nucleus as it is being forced into the pseudopodium, which is being thrust out. This may be repeatedly seen in living creatures. The nucleus itself is not really amoeboid, but undergoes passive distortion.

Encystment.—I have experienced great difficulty in finding any stages in this animal other than those just described. For a long time I could find no indications of encystment, in spite of trying all the means I could think of to bring it about. When I did discover the cysts, however, I came upon them in immense numbers, so that I was able to follow the process of encysting in great detail. All encysting forms were found in December, January and February (cf. the case of the flagellates), but this is perhaps merely a coincidence.

Before encysting, *Entamoeba* undergoes certain changes in its nucleus. The chromatin at the periphery increases in amount and is then gradually extruded¹ into the cytoplasm, where it lies in irregular masses (fig. 60). These masses gradually increase in size by the chromatin granules running together (figs. 61, 64, etc.) The process continues until quite a large quantity of chromatin is lying free in the cytoplasm. At about the same time the nucleus develops a karyosome at its centre. The karyosome always has the structure (seen in figs. 60, 61, 64) of a little heap of loosely packed granules. Fine filaments connecting it with the periphery can usually be distinguished (cf. fig. 60, etc.) The amoeba now slowly rounds itself off, and a large vacuole appears in the cytoplasm (fig. 61). When it has reached this stage the organism secretes a delicate cyst membrane (figs. 63, 64). In the living animal these cysts have a very characteristic and striking appearance, with their large nucleus, refractive chromatin masses, and big vacuole (fig. 63).

¹ I have not been able to watch this in the living animal. The statement is based upon a study of fixed and stained material.

Their size is variable; they measure from ca. $10\ \mu$ to $16\ \mu$ in diameter—on the average $12\text{--}13\ \mu$. The diameter of the nucleus is ca. $6\ \mu$.

The cysts remain in this uninucleate condition for some time—exactly how long I have not been able to determine. Then the nucleus begins to divide. In the living animal it is seen to grow out into a long spindle, which subsequently gives rise to the two daughter-nuclei, but no details can be seen. In stained preparations, however, the whole process of division can be followed out in every stage with extraordinary clearness (see figs. 65–70). The first thing observable is the formation of two little outgrowths at opposite poles of the nucleus (fig. 65). As these gradually draw apart the nucleus assumes the characteristic and remarkable spindle-figure (fig. 66). The karyosome lies at first in the middle of the spindle (fig. 65), but subsequently its component granules dispose themselves on the longitudinally arranged spindle fibres, as shown in fig. 67. At this stage the spindle extends almost from one side of the cyst to the other. Subsequent differentiation into two daughter-nuclei takes place through the rearrangement of the karyosomic granules to form two daughter-karyosomes, and through the subsequent constriction of the middle of the spindle (figs. 68, 69). When this is finally completed two daughter-nuclei, exactly like the original nucleus except that they are smaller, are seen inside the cyst (fig. 70).

In this binucleate condition the cyst remains for but a short time. Then both nuclei divide. They do not divide in quite the same way as the original nucleus. Instead of forming a spindle by outgrowth at opposite poles, as originally happened (fig. 65), they each form a spindle by outgrowth at one pole only (cf. figs. 71, 72, 73).¹ The karyosome therefore lies at

¹ It might be thought that these two nuclei (fig. 71) are not about to form the second spindles, but are just finishing the first division—the drawn-out poles being the points which were connected, as in the right-hand pair of nuclei in fig. 74. Many considerations render such an interpretation highly improbable. In the first place, in the living

one end of the spindle at the beginning of division (fig. 72), the "spindle" itself being really club-shaped. As the spindles draw out the karyosome granules travel along the spindle-fibres (fig. 73, lower spindle), and finally some of them reach the opposite end and give rise to the distal daughter-karyosome (fig. 73, upper spindle). Constriction of the spindle then takes place, as in the case of the first spindle, and finally four nuclei are formed in the cyst (figs. 74, 75). As will be apparent from the figures, the two secondary nuclei do not always divide simultaneously.

The next thing which happens is the removal of the chromatin masses in the cytoplasm. In many cases this is apparently absorbed, for it stains paler and paler, and gets smaller and smaller, and all fully-developed cysts are quite without free chromatin (fig. 77). But I have found a number of cysts which appear to indicate quite clearly that the chromatin is sometimes removed directly by extrusion from the cyst through the membrane (cf. fig. 76). Apparently, therefore, it may be got rid of in either way.

In addition to losing its chromatin masses the cyst now loses its vacuole (figs. 76, 77, 79). After this the cyst becomes slightly thicker and more yellow in colour. It contains the four nuclei (the product of the two nuclear divisions), each of which has a very characteristic appearance—that of a ring with a central karyosome granule (fig. 77). The structure of the nuclei can be seen quite clearly in the living cyst (fig. 79). Each nucleus measures about $3\ \mu$ in diameter—that is, half the diameter of the original single nucleus which was present in the cyst (fig. 64).

animal the spindles are seen to grow across from one side of the cyst to the other. Then, again, we should expect to find the projections turned towards one another if they were the results of the first division. But actually they are often directed in opposite directions (cf. fig. 71). It should also be noted that the spindles of the second division remain pointed at only one end until quite late in development (cf. both spindles in fig. 73). All the facts are in favour of the interpretation given above.

When the cysts reach this stage they cease to develop. I have never found cysts containing more than four nuclei.

The cysts remain in this condition for many days if left in water. If dried they invariably die. But even in the water a large proportion of cysts always degenerated, gradually breaking up (fig. 78).

Attempts to cause the cysts to develop further in the intestinal juices of frogs have always been negative—as I believe, owing to the abnormal state of the frogs used (cf. pp. 213, 264).

If we compare the nucleus of the smallest kind of amœba found in the frog (fig. 54) with the nuclei in the fully-formed cyst (fig. 77) we cannot fail to be struck by their similarity. They correspond closely in structure and size. It appears to me probable that when the cyst reaches its new host's gut its contents break up into four small amœbæ, which are then set free and grow up into the ordinary form, just as in *Entamœba coli* the cysts liberate broods of eight (Schaudinn [43]).

Perhaps it may also be inferred, from the kind of chromatin reduction which takes place during encystment, that the four nuclei are reduced gamete nuclei, and the small amœbæ liberated from the cysts conjugate with one another. But this is mere hypothesis. The history of the chromatin is, at all events, suggestive.

Now it must be apparent to anyone reading this description and looking at the figures that at certain stages of development there is an extraordinary resemblance to certain stages in the autogamy of *E. coli* (Schaudinn [43]) and *E. muris* (Wenyon [87]). Indeed, had one encountered isolated stages, and had one not been able to follow up every stage of development, one would be strongly inclined to believe that an autogamy occurred also in *E. ranarum*. (Compare some of the figures in Pl. 4 with those of Wenyon, e. g. the cyst with two spindles [fig. 72], with a similar cyst [fig. 23, Pl. 10] of Wenyon's paper). I do not for a moment suggest that Schaudinn and Wenyon were guilty of misinterpreting

what they saw. I admire greatly the work of both. I merely draw attention to the resemblance.

I have even found stages which might at first sight be thought to show a condition in which the nucleus was being completely analysed into chromidia (fig. 62). From comparison of the "chromidia" with the micro-organisms in the same preparation, and also from what I have seen in the living animal, I have no hesitation in saying that the "chromidia" are really bacteria, and we are here dealing with a case of bacterial invasion, in which the nucleus is attacked as well as the cytoplasm. The animals appear particularly liable to the attacks of bacteria just before forming the cyst membrane.

From what I have already said it will be apparent that I can confirm neither the statements of Brass as regards spore-formation, nor those of Hartmann¹ regarding autogamy in *E. ranarum*. On the contrary, I have found that the nucleus undergoes a perfectly straightforward series of changes leading up to the formation of a quadrinucleate cyst, which probably serves for the dissemination of the organism.

The nuclear divisions in *Entamoeba ranarum* present some interesting features. Division does not seem to correspond with any of the forms hitherto described. Mitosis was first described in an *Amoeba* by Schaudinn (78), and he also gave (76) the first accurate description of amitosis in the genus. Similar observations have been made on other forms by other observers since. The nuclear division I have just

¹ After writing the foregoing remarks I received Hartmann's paper on an amoeba (*Entamoeba tetragena* Viereck = *E. africana* Hartmann) found in certain cases of dysentery in man. Hartmann believes that an autogamy occurs, but from his figures I have little doubt that future observations on the living animal will show that a condition almost identical with that seen in *E. ranarum*—as already described in preceding pages—really prevails. Hartmann's figures bear an extraordinary resemblance to isolated stages in the development of *E. ranarum*. (See Hartmann, *Beih.* 5, 'Arch. Schiffs. Tropenhygiene,' xii, 1908.

described appears to be neither truly mitotic nor truly amitotic, but rather of an intermediate type.

The difference between the method of formation of the first spindle and that of the second pair in the cysts is as extraordinary as it is unaccountable. I cannot suggest even the slightest reason for it.

In addition to the parasitic amœba found in the intestines of frogs and toads, one sometimes meets with another amœboid organism, which differs in many respects from that just described. As a result of culture experiments with the contents of the intestine, I am now convinced that this organism represents a phase in the life-history of the shelled rhizopod, *Chlamydophrys stercorea* Cienkowski, which I will now describe. I may mention also, en passant, that minute *Amœbæ* belonging to the limax-group also turn up frequently in cultures made from the fæces. But then they are found quite commonly in organic infusions of almost any kind. Still, their presence, and that of leucocytes, offer difficulties to the investigator, and for that reason I mention the fact.

(2) *Chlamydophrys stercorea* Cienkowski.

Syn.: [? *Diffflugia enchelys* (Ehrbg.) Cienkowski, 1876].

Troglodytes zoster Gabriel, 1876.

Platoum stercoreum (Cienkowski) Bütschli, 1880.

Leydenia gemmipara Schaudinn, 1896.

Chlamydophrys stercorea (Cienkowski) Schaudinn, 1903.

This very interesting rhizopod was first described and named by Cienkowski in 1876 (5). He says it is the same organism that Schneider¹ described under the name *Diffflugia enchelys* Ehrbg., but I think there can be no doubt that it is not. *D. enchelys* Ehrbg., is really the same as *Trinema*

¹ 'A. Schneider, 'Muller's Arch. Anat. Physiol.,' 1854, p. 191.

acinus Duj., described by Dujardin, Claparède and Lachmann, F. E. Schulze, and others (see their descriptions and figures). In the very same year that Cienkowski's work appeared a remarkable account of an organism, named *Trogloodytes zoster*, was published by Gabriel (19). From his description I feel almost convinced that he really observed the same organism. This work is remarkable in that it anticipates the discovery of many of the stages in the life-history of this animal, with which we have since become acquainted through the labours of Schaudinn (43). Unfortunately Schaudinn's full description never saw the light, so that for the present our knowledge rests upon his lucid but brief preliminary paper. Many points still require confirmation, therefore; for instance his statement of its identity with *Leydenia gemmipara* Schaud., the amœboid organism described by Leyden and Schaudinn (36) in ascitic fluid. Taking the results of Cienkowski, Gabriel and Schaudinn together, we appear now to have a fairly perfect knowledge of the life-cycle of *Chlamydothryx*. Nevertheless, as the work requires confirmation I think my observations may not be superfluous.

According to Bütschli (3) *Chlamydothryx* is a synonym for *Platoun* F. E. S. But this is really a free-living form, similar to, but not the same as, *Chlamydothryx*.

As is well known, *Chlamydothryx* is an animal which lives in the fæces of various animals, the cysts passing along the alimentary tract before they undergo development. It is still unknown whether the forms of *Chlamydothryx* found in the fæces of different animals represent one species or several. This can be decided only by further research.

I have found the organism in *Rana temporaria* and *Bufo vulgaris*, but not frequently. In both the animal appears to be identical.

Schaudinn was the first to observe that the animals might escape from their cysts in the form of an amœba before leaving the intestine of the "host." This happens only occasionally.

The *Chlamydothryx* amœbæ, which I have found in the

large intestine or in the discharged excrement of frogs, have a very characteristic appearance (see Pl. 5, figs. 81, 82). They are active and show a well-marked ectoplasm and endoplasm (fig. 81). The protoplasm itself often shows a most striking alveolar structure, which is no less marked in the living animal than in a fixed and stained specimen (compare figs. 81 and 82). In the living state also the structure of the nucleus is seen quite as distinctly as in a stained preparation. It is characterised by a large central mass of chromatin, separated by a clear zone from the membrane. Its structure corresponds very well with that figured in "*Leydenia*" by Schaudinn. It is impossible to mistake this amœba for *Entamœba ranarum* (compare figs. 81 and 52). A contractile vacuole is sometimes, but not always, to be seen. When present it can often be seen to arise as several small vesicles, which fuse together during the diastole.

Although the amœbæ are sometimes to be found in large numbers I have never succeeded in finding dividing forms. According to Schaudinn they are capable of multiplying both by equal bipartition and by budding.

It is interesting to note that they will live and apparently multiply—though I have never found dividing individuals—in saline albumen solution. On one occasion when I transferred some amœbæ into a culture dish containing the albumen solution, I found that after the lapse of twenty-four hours they had increased considerably in numbers and were very active. Owing to drying of the solution many of the amœbæ subsequently encysted. This ability to live thus is of interest in connection with Schaudinn's statement that "*Leydenia*" is really the *Chlamydomphrys* amœba.

After creeping about in the fæces for some time the amœbæ come to rest and develop into the typical adult form. They do this by rounding themselves off and developing a shell—a thin, shining, white, porcellaneous structure. It is oval in shape, with the opening at the apex (see fig. 80, Pl. 5). Through the opening, the animal protrudes delicate filose pseudopodia, which serve to catch its food.

A good deal of variation is seen in the size of the animals. The measurements correspond very closely with those given by Gabriel for "Troglodytes." An average large-sized individual measures about $20\ \mu$ by $14\ \mu$.

Inside its shell the animal's body is roughly differentiated into two zones—an anterior vacuolate zone, lying immediately behind the shell aperture, and a posterior non-vacuolate zone containing the nucleus. As the food particles reach the interior through the shell opening they appear to be digested entirely in the vacuolate zone. In this zone contractile vesicles are also to be found sometimes. Their number varies, and they are not always present. No distinct partition into two zones—as in *Diffugia*, *Euglypha*, etc., occurs.

According to Schaudinn the nucleus is surrounded by a chromidial mass, which subsequently breaks up to form the gamete nuclei—usually eight in number. I have never been able to find a distinct chromidial net, though the protoplasm in the posterior region is often very dense, and contains many darkly-staining granules. Possibly these are the chromidium in its early stages. Later stages, with gamete formation, have never come under my notice.

The nucleus itself is precisely the same as in the free-living amœboid form. Occasionally more than one nucleus is present, as Cienkowski long ago noticed.

When the cultures containing *Chlamydophrys* were allowed to dry the animals very readily encysted. This happened even before the animal had developed its shell. But the shelled forms also encysted, the protoplasm flowing out of the shell-opening becoming spherical and secreting its cyst wall outside. The shells subsequently broke up. On one occasion I found an animal which had encysted inside its shell (fig. 84), but this is very unusual.

The cysts themselves (fig. 83) vary enormously in size. The smallest are about $6\ \mu$ in diameter, the largest 16 – $17\ \mu$. An average size is $14\ \mu$. Their most striking feature is their thickness, which is often very great in places. They are

very irregular externally and always heavily encrusted with foreign bodies (cf. fig. 86). Their colour is brown or brownish-yellow. They are not unlike those of *Copromonas*, though usually to be distinguished by their excrescences.

As a rule only one nucleus can be seen in each cyst. Once, however, I found a cyst containing two individuals, each with a nucleus (fig. 85). This is rare.

Schaudinn found that, in order to develop, the cysts had to pass through the alimentary canal of the "host" animal. But this is not the case with the *Chlamydophrys* from frogs. Perhaps the cysts I observed were only temporary, and not the same as the durable structures which arise after conjugation. At all events, I found that moistening the dried fæces sufficed to cause a number of animals to emerge from their cysts. It appears to be immaterial whether the fæces are moistened with salt-solution, water, or juice from the intestine. In each case the cyst-wall dissolved, and the animal emerged and began life once more as an amœba. (See Pl. 5, figs. 87-90, which show emergence of an amœba from a cyst.) A considerable percentage of cysts never dissolved. A good many showed protoplasmic streaming after the addition of liquid to the fæces, but after a time it ceased and the cysts showed no further signs of life.

At the time when I encountered *Chlamydophrys* most abundantly I was unfortunately so busily engaged in working at other forms that I was unable to take proper care of the cultures. The result is that what few further observations I was able to make, though interesting and curious, were too uncertain to carry much weight. In consequence I cannot add anything more to the account of the life-history already given by Schaudinn.

I may remark that the "*Amœba* sp." which Wenyon describes in the mouse, in addition to *Entamœba muris*, is perhaps also *Chlamydophrys*, or an allied form.

C. SPOROZOA.

In my preliminary note (12) I recorded the presence of a new coccidian in the intestine of the frog. I then gave the name *Coccidium ranæ* to the organism. But having since made a more careful study of the literature, I am reluctantly compelled to relinquish the generic name *Coccidium* in favour of the apparently more accurate *Eimeria*. That a long-familiar name like *Coccidium* should have to be completely abolished is indeed deplorable. I feel convinced, however, that the retention of this name (as Schaudinn and Minchin have retained it) is really unjustifiable. Unless our system is to be thrown into absolute disorder there can be no place for anarchy in zoological nomenclature—whatever one's feelings in the matter may be. The name of this coccidian is therefore—

Eimeria ranæ Dobell.

A few words must first be said to justify the specific distinction given to this animal, as several coccidian parasites have already been recorded in frogs. The history of these is briefly as follows:

Liebkühn (37) was the first to describe "psorosperms" in frogs. He found these in the kidneys only, not in the intestine. The parasite described by him is that now known as *Isospora liebkühni* Labbé. In 1870 Eimer (17) found in frogs the developmental forms of a coccidian, which he considered was probably the same as that which he observed in the mouse (*Eimeria falciformis* Eimer¹). Pachinger (41) also found intestinal coccidia—in the duodenum of *Rana esculenta*. He gave the name *Molybdis entzi* to them, but gave an insufficient description of their structure. It is thus impossible to know whether the parasites described by Eimer and Pachinger correspond with my form or not.

¹ Called by him "*Gregarina*" *falciformis*, however.

Labbé (30) mentions that he found a parasite, like that occurring in newts, in the nuclei of the intestinal epithelium of *Rana temporaria*. Without giving any further description he bestows the name *Karyophagus ranarum* n. sp. upon it. But on the very next page (p. 212) he says that he believes that this parasite is identical with *Karyophagus salamandræ* Steinhaus and *Cytophagus salamandræ* Steinhaus. And he proposes to call them all *Acystis parasitica*! Later (31) Labbé retains the name *Caryophagus ranarum* Labbé for the intestinal coccidian of the frog, but gives the host as *Rana esculenta*, and gives no further description of it. It is obviously useless to attach much importance to these names, and impossible to identify the animal.

The only careful work which has been done upon the coccidian parasites of frogs is that of Laveran and Mesnil. But none of the forms described by them appear to correspond with my form. These two investigators have worked out the whole of the life cycle of the organism found by Lieberkühn, and have discovered some very interesting details (Laveran et Mesnil [32]). Of special interest is the fact that this parasite, though normally attacking the kidneys, may give rise to a general infection of the host. And in such cases the small intestine may be infected. However, this animal has nothing to do with the form under consideration: it is an *Isospora*, with disporic oocyst and tetrazoic spores. Laveran and Mesnil described also (33) two more coccidians (from *Rana esculenta*), giving them the names *Coccidium ranarum* and *Paracoccidium prevoti*. The latter differs from all other coccidia in that the sporocyst dissolves in the later stages of development, so that the sporozoites come to lie freely in the oocyst. The former, however, presents many points of resemblance with my parasite. But it differs in several points, the most important being the absence of an oocystic residuum. Quite recently Mesnil (38) has found another coccidium—an *Isospora*—in the gut of *Hyla arborea*.

It thus appears to me certain that the parasite under

discussion has not previously been described, and must hence be made a new species.

With regard to the occurrence of *Eimeria ranæ*, I can record the facts that it was found in *Rana temporaria* (Cambridge and Munich), in ca. 15 per cent. of all frogs examined,¹ and upon a single occasion in *Rana esculenta* (Munich).

I have always encountered the stages of the sporogony of this organism in the lower end of the frog's gut—about the posterior half of the small intestine, together with the large intestine. Although I have cut a large number of sections and made repeated examinations of the epithelium of the small intestine and the liver, both in frogs containing spores and in those apparently uninfected, I have never succeeded in finding the slightest trace of the schizogony. I have also examined the kidneys without result, but the distribution of the spores seems to exclude the possibility of these being the seat of schizogony. It appears most probable that schizogony takes place in the small intestine—in the upper part—and is completed before any of the parasites proceed to spore formation. Hence the presence of oocysts in the rectum indicates that schizogony is finished.

Sporogony.—I have been able to follow the whole of the sporogony of this coccidian in the living animal. I have completely failed to obtain stained preparations at any stage, owing to the extraordinary impermeability of the oocysts and spores. Every method has proved unavailing. Even the methods which I have found successful in other cases—dilute acid Delafield's hæmatoxylin, acid alcoholic carmine, etc.—have quite failed in this case. I have left the oocysts, etc. in these stains for over two months without any staining whatsoever taking place. The following is the series of stages to be seen in the living organisms:

The earliest stage found is that shown in Pl. 5, fig. 92. The oocyst is already well developed, and the contents of the cyst are seen to consist of a dense mass of very highly

¹ But it occurs more frequently in the Cambridge frogs.

refractive bodies (reserve material). The cysts are spherical or somewhat ovoid and measure $18-22\mu$ in diameter. Occasionally—as in fig. 92—a clear area can be seen in the centre, in optical section. This is probably the nucleus.

If such a cyst be kept under observation for some time it is seen gradually to undergo internal changes. The contents slowly become divided up into five masses—at first irregular, but subsequently recognisable as four sporoblasts and an oocystic residuum (fig. 93). This process of segmentation is very slow, and takes from about twelve to twenty hours for completion. No nuclear changes were ever made out.

The changes which now ensue concern the metamorphosis of the sporoblasts into spores. At first the sporoblasts are spherical, with a diameter of about 7.5μ (fig. 94). In course of time they become oval, however, measuring about 10μ by 7μ . They then begin to show a clear area of protoplasm at one spot, quite free from the refractive bodies (fig. 95). The time taken to reach this stage is about another twenty hours or so after the spherical sporoblasts are clearly differentiated.

Subsequently the sporoblasts slowly change into spores. They acquire a membrane, and later begin to have a “pseudo-navicella”-like appearance (fig. 96). Inside the developing spore the refractive bodies heap themselves into a spherical mass, which later represents the sporal residuum. This stage is reached after about another six to seven hours.

From this stage onward development proceeds more slowly. The spore-membrane thickens, acquiring a very evident double contour, with a knob-like eminence at either end. The spores are now markedly “pseudo-navicella”-like in shape (fig. 97). Inside the spore the clear protoplasm is very sharply marked off from the residual mass, which now lies centrally. The clear protoplasm can be seen gradually to become divided in a longitudinal direction into two sporozoites, which lie with their ends curled over one another, tête-bêche (fig. 97). With careful arrangement of the illumination it can be seen that each sporozoite has a nucleus situated towards the middle of its body (fig. 97). They lie

quite motionless inside the spores. When the latter are fully formed the oocyst usually collapses over them (fig. 91), so that they remain loosely encapsuled together. Sometimes the oocyst completely breaks up if kept in a liquid medium, and the spores then become free. They measure ca. 14μ by 7μ . Their resemblance to the spores of *Monocystis* is often very striking in early stages of development. As I have already noted (p. 206), these spores are not uncommon in frogs. Of course, when fully formed the octozoic *Monocystis* spores cannot possibly be mistaken for the dizoic spores of the *Eimeria*.

As in all the other forms investigated, I have found great difficulty in causing the contents to emerge. The gastric juice of the frog is quite without action upon the spores. So also are 2 per cent. HCl, 3 per cent. Na_2CO_3 , and artificial solutions of trypsin or pepsin. The juices from the small intestine of laboratory frogs is also, as a rule, without effect. On a single occasion, however, when I used the juice from the upper part of the small intestine of a frog killed almost immediately after it was captured, I saw the following events take place: In three or four spores lying near to one another, the sporozoites—after about a quarter of an hour—began to move about inside their spores. The movements increased, and finally the sporozoites were seen in a state of great activity, chasing one another round and round inside their narrow prison, jostling the residual mass. After this had continued for another hour one spore suddenly burst and a sporozoite emerged. But it then, almost at once, ceased to move, and, after swelling up, died and broke into fragments. The other sporozoites all became motionless subsequently, and none of them came out of their spores. All other attempts to get them to emerge have been fruitless.

I think this experiment indicates that the spores are probably dissolved, and the sporozoites emerge, in the upper part of the small intestine of the frog. The reason that experiments are nearly always negative is probably to be sought in the changes which the digestive juices undergo in

frogs kept in the laboratory. The juices seem to become inactive after the frogs have been kept in captivity without receiving their usual food.

Metzner (67) has already pointed out that the spores of *Eimeria stiedæ* are opened by pancreatic and not gastric juice—a condition which probably obtains also in *E. ranæ*.

In conclusion, I may call attention to the similarity which exists between the sporogonic stages of this coccidium and those of *Eimeria salamandræ*, Steinh. (see Simond's figures, etc.). The schizogony and fertilisation of this animal are now known, through the work of Steinhaus, Simond and others (cf. 83, 82, 81, 14).

D. CILIATA.

I am able to add but little to the life-history of the Infusoria which occur in the frog. The two following observations, however, appear to me worth recording.

(1) Encystment of *Nyctotherus cordiformis* Ehrbg.

As far as I know the cysts of this animal have not been described hitherto: which is not surprising, as they are exceedingly rare. When removed from the frog *Nyctotherus* nearly always dies.

The cyst is shown in Pl. 3, fig. 51. It is a more or less oval structure, between 80 μ and 90 μ in length (that figured measured 87 μ). Its colour is greenish-yellow, and it shows a very distinct striation, the striæ following the lines in which the cilia were disposed in the free animal. The mouth and gullet can be seen, though somewhat indistinctly. The meganucleus is very distinct, but I have never been able to distinguish a micronucleus with certainty. One or more vacuoles may be present. They continue to pulsate for some time after the cyst has been formed.

I have kept the cysts in water for over a week, but they finally died. They do not seem able to withstand drying.

(2) Culture of *Balantidium* entozoon Ehrbg.

I have been able to watch division and encystment in this animal on many occasions, but these have been already described by others in more or less detail. The following observations are of interest in relation to the species question—many different vertebrates harbouring a *Balantidium* resembling *B. entozoon*.

I have found that it is possible to cultivate this organism in infusions made from the faeces of a variety of different animals (rats, snakes, etc.). The interest lies in the fact that they not only survive, but also remain extraordinarily active and multiply by frequent division. They will continue to do this for days, so that it is thus possible for these parasites to live and increase also as saprophytes.

I have found cysts in these cultures, but whether they were formed there or originally introduced I cannot say.

It may be added that *Balantidium* can also exist in certain organic infusions for a considerable time.¹

In conclusion, I gladly take this opportunity of offering my warmest thanks to Professor Richard Hertwig for his kindness to me whilst working in the Zoological Institute in Munich. I wish also to thank Dr. Richard Goldschmidt for the friendly interest he took in my work whilst there, and for his readily offered advice. But my greatest debt of gratitude is owing to Professor Adam Sedgwick. What measure of success I have achieved is due largely to his inspiration and encouragement—without which I should never have undertaken these researches. I desire, therefore, to thank him most sincerely, as some slight acknowledgment of my indebtedness.

¹ Walker has quite recently published an account ('Journ. Med. Research,' xviii, 1908) of successful cultivation experiments made by him on the flagellates and ciliates in frogs. He states that he has been able to cultivate *Nyetotherus*, *Trichomonas*, and "*Cercomonas*" (? *Octomitus*) on agar media. Neither he nor Strong ('Bull. Gov. Lab.,' Manila, 1904), however, has succeeded in cultivating *Balantidium coli*. For my own part, I have not been able to cultivate the flagellates of frogs on Musgrave and Clegg's medium for more than a few days.

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ZOOLOGICAL LABORATORY, CAMBRIDGE;
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EXPLANATION OF PLATES 2—5,

Illustrating Mr. C. Clifford Dobell's paper on "Researches on the Intestinal Protozoa of Frogs and Toads."

PLATE 2.

[Figs. 11 and 12 are drawn from living animals. The remainder are from permanent preparations. Fixation: hot sublimate-alcohol (Schaudinn). Stain: Heidenhain's iron-hæmatoxylin. Figs. 1, 2, 5, 7, 8, 9 and 21 counterstained with Bordeaux red. All drawings made under Leitz $\frac{1}{12}$ in. oil-immersion with ocular No. 5.]

Figs. 1-15.—*Trichomastix batrachorum*.

Fig. 1.—Ordinary vegetative individual, showing general structure.

Fig. 2.—Form with thick axostyle. The attachment of the blepharoplast to the bent axostyle is very clearly seen.

Fig. 3.—Form with very slender axostyle.

Figs. 4-12.—Stages in division.

Fig. 4.—First stage in division. The axostyle has disappeared, there is no nuclear membrane, and the blepharoplast is beginning to divide.

Fig. 5.—The blepharoplast is now elongated, forming a rod with two flagella at either end.

Fig. 6.—The chromatin is arranged in a spindle-shaped mass of small granules, and new flagella have made their appearance. (The young flagella are by no means always so well developed as in this instance.)

Fig. 7.—The chromatin is now arranged in large lumps, and the outgrowth of new flagella is very clearly seen.

Fig. 8.—At this stage the chromatin has travelled in two irregular masses towards the blepharoplasts.

Fig. 9.—A similar stage to the preceding. The rod connecting the daughter-blepharoplasts is very distinctly seen.

Fig. 10.—The large lumps of chromatin have broken up to form the new nuclei of the daughter-monads. A thick rod lies between the two nuclei.

Fig. 11.—A somewhat later stage.

Fig. 12.—The same creature a few seconds later. The protoplasm, after welling in and out rapidly several times, has suddenly been constricted, completely severing the two daughter-monads. Each monad has an axostyle which is half of the rod-like structure which connected the blepharoplasts.

Fig. 13.—*Trichomastix* which has developed a karyosome in its nucleus and is preparing to encyst.

Fig. 14.—Newly-encysted animal. The flagella have gone and the axostyle is degenerating.

Fig. 15.—Permanent cyst. The axostyle has quite disappeared and the nucleus has taken on its characteristic elongate form, with the blepharoplast lying upon it.

Figs. 16–28.—*Trichomonas batrachorum*.

Fig. 16.—Ordinary individual, large specimen.

Figs. 17–24.—Stages in division.

Fig. 17.—First stage in division. The axostyle has gone, and the edge of the undulating membrane has split.

Figs. 18, 19, 20, 22, 23, 24.—Various stages in division, corresponding with those shown in *Trichomastix*. (Cf. figs. 4–10.) The membrane is seen in various stages.

Fig. 21.—In this organism a very distinct spindle figure is seen during the division of the nucleus. Note also the diplosomic blepharoplasts and undulating membranes.

Fig. 25.—*Trichomonas* about to encyst. Nucleus with karyosome.

Figs. 26, 27.—Two successive stages in encystment. Resorption of axostyle, undulating membrane, etc.

Fig. 28.—Permanent cyst, with elongate nucleus and no axostyle or locomotory organellæ.

PLATE 3.

[Figs. 31, 39, 42, 43, 44, 45, 47, 48 and 51 are drawn from living specimens under Zeiss 2.5 mm. apochromatic water-immersion, comp. oc. 12. Remainder from permanent preparations: figs. 46, 49 and 50 fixed absolute alcohol, stained Giemsa; the others sublimate-alcohol and Heidenhain's iron-hæmatoxylin. Drawn, unless otherwise stated, under Leitz 2 mm. oil-immersion (apochrom.) with comp. oc. 12.]

Figs. 29–41.—*Octomitus dujardini*.

Fig. 29.—Ordinary individual to show nuclear apparatus, etc.

Fig. 30.—Individual with more consolidated nucleus, crossed axostyles, etc.

Fig. 31.—Living animal, moving slowly—axostyles parallel.

Figs. 32–35.—Various small forms, showing different forms of nucleus, etc. (Zeiss 1·5 mm. apo. oil-imm., comp. oc. 12.)

Figs. 36, 37.—Degenerate forms.

Fig. 38.—Encysted *Octomitus*.

Fig. 39.—Individual moving about rapidly inside cyst. Drawn just before emerging.

Figs. 40, 41.—Probably represent stages in division.

Fig. 42.—*Bodo* sp. form faeces of *Bufo vulgaris*.

Fig. 43.—Another individual, amœboid at hind end.

Fig. 44.—Extremely minute *Bodo* individual.

Fig. 45.—Cyst containing four organisms—probably *Bodos*.

Fig. 46.—A six-flagellate organism from faeces of *Bufo*. (Zeiss 3 mm. apo. oil-imm., comp. oc. 12.)

Fig. 47.—Minute unflagellate monads from faeces of toad.

Fig. 48.—Two three-flagellate monads, also from faeces of toad.

Figs. 49, 50.—*Monocercomonas bufonis*—wide and narrow forms. (Zeiss 2 mm. apo. oil-imm., comp. oc. 12.)

Fig. 51.—Cyst of *Nyctotherus cordiformis*. (The pale, sausage-shaped structure is the nucleus; the smaller, light area, above to the left, is a vacuole. Below this is to be seen the rather faint outline of the mouth and gullet.)

PLATE 4.

[All drawings, unless otherwise stated, are made from permanent preparations, fixed with sublimate-alcohol, and stained with Delafield's hæmatoxylin. Drawings made (unless stated to the contrary) under Zeiss 3 mm. apochromatic homog. oil-immersion (1·40), comp. oc. 12.]

Figs. 52–79.—*Entamœba ranarum*.

Fig. 52.—Large vegetative individual. Living animal. The nucleus is seen as a very distinct ring-like (in optical section) structure, with a beaded appearance, lying near the centre, surrounded by food masses. (2·5 mm. apo. water-imm. [Zeiss] × c. oc. 12.)

Fig. 53.—Ordinary individual. The typical appearance of the nucleus in a stained specimen is well seen.

Fig. 54.—Smallest kind of amœba found, with characteristic nucleus containing a small karyosome. (Formalin 40 per cent., Heidenhain iron-hæmatox.)

Fig. 55.—Individual with nucleus in process of dividing. (Leitz $\frac{1}{12}$ in. oil-imm. \times oc. 5.)

Fig. 56.—Amœba with two nuclei—presumably in a stage just before fission of cytoplasm. (Heidenhain Fe-hæmatox. Leitz $\frac{1}{12}$ in. \times oc. 5.)

Fig. 57.—Individual with distorted nucleus. The distortion is brought about by the nucleus being forced into a pseudopodium. The condition suggests—falsely—an amitotic division. (Delafield and eosin. Leitz $\frac{1}{12}$ in. \times oc. 5.)

Fig. 58.—Large, actively feeding amœba, with modified nucleus. (Hypertrophied; drawn on smaller scale than other figures. Heidenhain and eosin, Zeiss 2 mm. apo. oil-imm., comp. oc. 6.)

Fig. 59.—Nucleus of same individual more highly magnified (comp. oc. 12.)

Fig. 60.—Amœba about to encyst. Note formation of karyosome and protrusion of chromatin into the cytoplasm (on left of nucleus).

Fig. 61.—Encysting amœba. The karyosome is now very well formed, the chromatin masses are very conspicuous in the cytoplasm, and the vacuole has made its appearance. No cyst membrane is as yet to be seen.

Fig. 62.—An amœba, at a similar stage, which has been invaded and killed by bacteria. These have filled the cytoplasm and attacked the nucleus, thus giving rise to an appearance which suggests a resolution of the nucleus into chromidia. (Heidenhain.)

Fig. 63.—A uninucleate cyst, living animal. Nucleus, chromatin masses, and vacuole well seen. (Leitz 2 mm. oil-imm. apo., comp. oc. 12.)

Fig. 64.—A similar cyst, stained.

Fig. 65.—Nucleus beginning to divide.

Figs. 66, 67.—Two succeeding stages of the spindle figure of the first nuclear division. (Fig. 67, Heidenhain and eosin.)

Fig. 68.—Later stage, in which the spindle is being constricted into two.

Fig. 69.—Still later. The two daughter-nuclei are now clearly differentiated, but not yet separated, owing to a part of the spindle persisting between them. (Heidenhain and eosin.)

Fig. 70.—Cyst containing two nuclei, formed by the division of the original nucleus. (Heidenhain and eosin.)

Fig. 71.—The two nuclei beginning to form the spindles of the second nuclear division. Note the way in which the spindle is being formed by extension of only one pole—not by prolongation between two opposite poles (cf. fig. 65). (Heidenhain and eosin.)

Fig. 72.—Cyst with two fully-formed nuclear spindles.

Fig. 73.—Later stage. The upper spindle is further advanced than the lower.

Fig. 74.—Final stage in second nuclear division.

Fig. 75.—Cyst with four nuclei, after second nuclear division. Compare nuclei—as regards size and structure—with those in figs. 64 and 70. (Heidenhain and eosin.)

Fig. 76.—Extrusion of chromatin masses. The vacuole has now disappeared. (Heidenhain.)

Fig. 77.—Cyst after extrusion of chromatin and collapse of vacuole. The membrane is thicker and yellowish. Four very distinctly outlined nuclei are seen. Compare their structure with that of nucleus in fig. 54.

Fig. 78.—Degenerating cyst, with four nuclei.

Fig. 79.—Living 4-nucleate cyst—same stage as fig. 77. (Leitz $\frac{1}{2}$ in. oil imm. \times oc. 5.)

PLATE 5.

[Figures, unless otherwise stated, drawn from living animals, under Zeiss 2.5 mm. apo. water-immersion, comp. oc. 12.)

Figs. 80-90.—*Chlamydomphrys stercorea*.

Fig. 80.—Shelled *Chlamydomphrys*, in fæces of toad. (Formalin 40 per cent. Heidenhain iron-hæmatox.)

Fig. 81.—Amœba stage of *Chlamydomphrys*, from large intestine of toad.

Fig. 82.—Similar organism. (Sublimate-alcohol, Heidenhain).

Fig. 83.—Cyst—encrusted, and with a single nucleus. (Formalin 40 per cent. Heidenhain.)

Fig. 84.—An animal which has encysted inside its shell.

Fig. 85.—Cyst containing two individuals. (Sublimate-alcohol, Heidenhain.)

Fig. 86.—Cyst, more highly magnified. (Comp. oc. 18). Note the peculiar knobs or excrescences on the outside, and the thick encrustment of bacteria, etc.

Figs. 87-90.—Four stages in the development of an amœboid *Chlamydomphrys* from its cyst, after moistening the dried-up fæces with water. (Comp. oc. 6.)

Fig. 91-97.—*Eimeria ranæ*.

Fig. 91.—An oocyst containing four spores and a residuum.

Fig. 92.—An oocyst (undeveloped) from small intestine of frog.

Fig. 93.—The same oocyst twenty hours later. It contains now four sporoblasts and a residuum.

Fig. 94.—A single sporoblast, more highly enlarged.

Fig. 95.—The same, twenty-one hours later.

Fig. 96.—The same—now becoming a spore—seven hours later.

Fig. 97.—The same—now a fully formed spore—thirty-six hours later. Two sporozoites and a sporal residuum are seen inside the “pseudo-navicella”-like spore.





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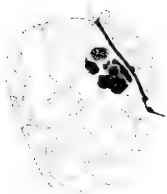
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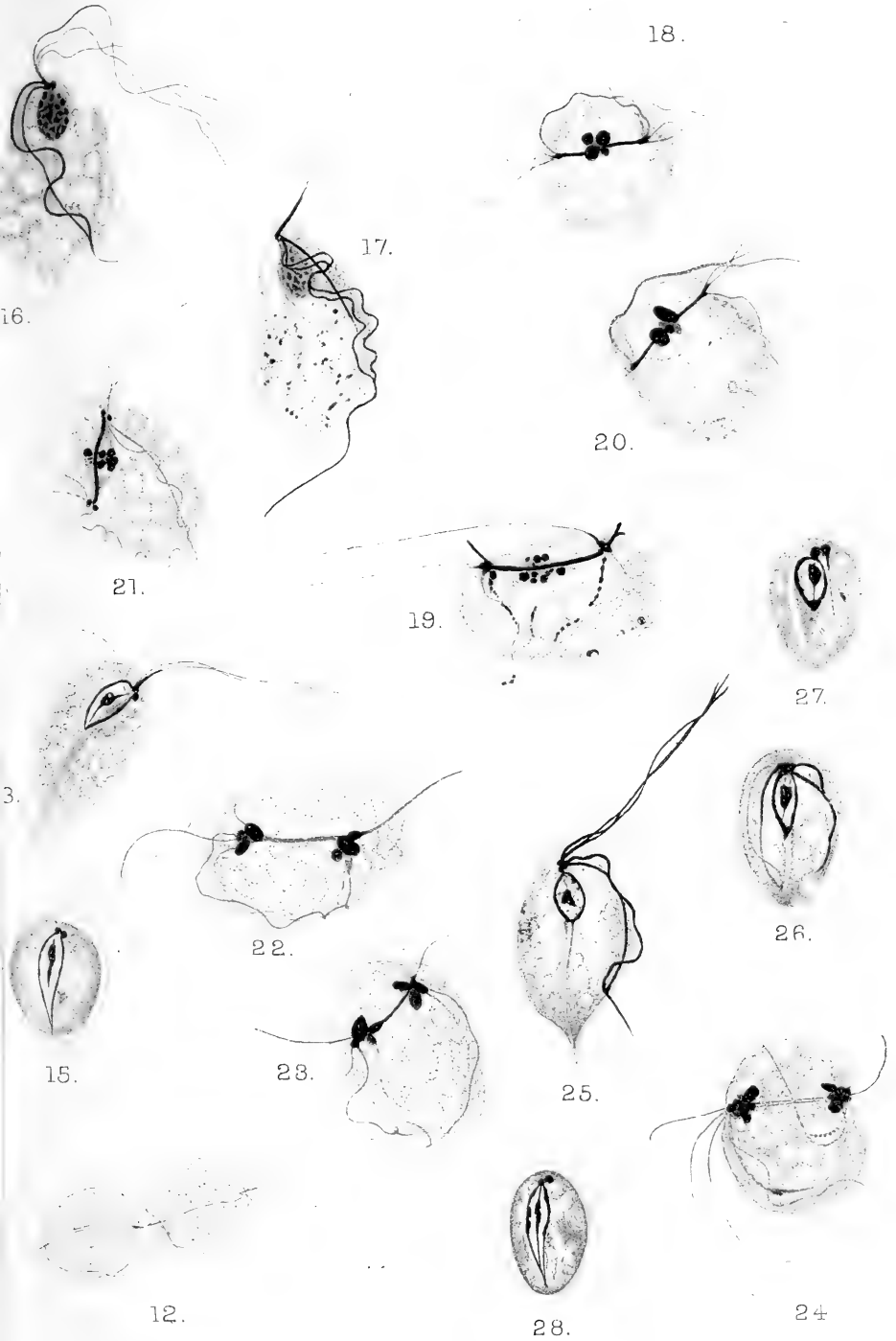
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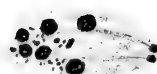
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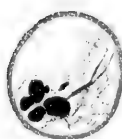
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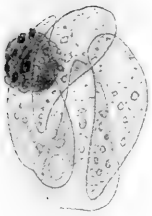
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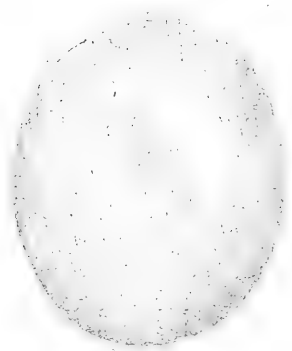
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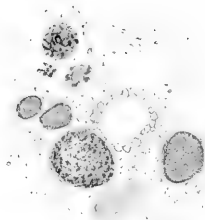


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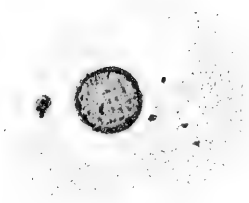


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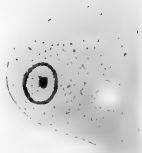




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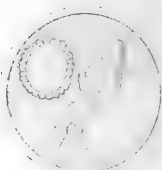
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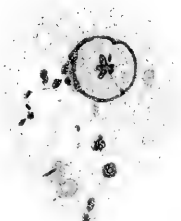
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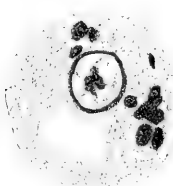
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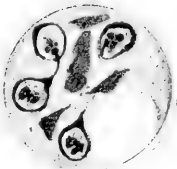
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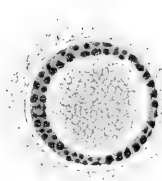
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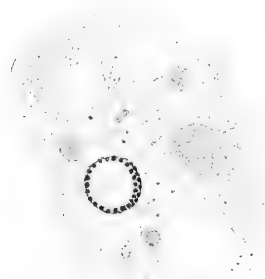
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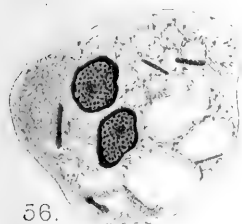
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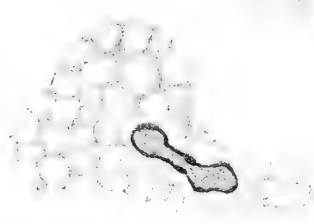
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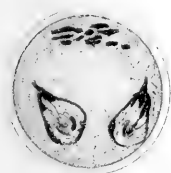
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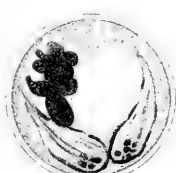
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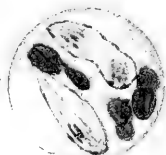
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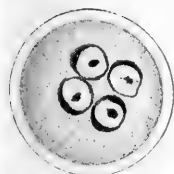
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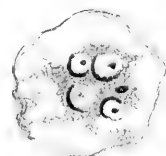
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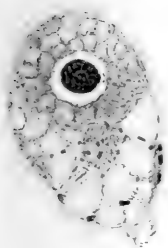
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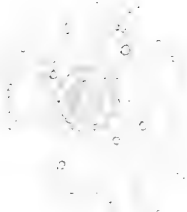
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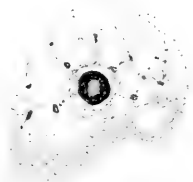
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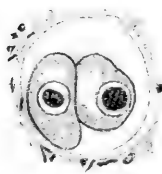
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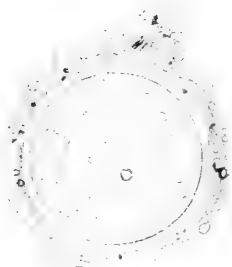
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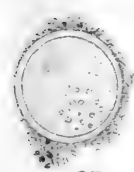
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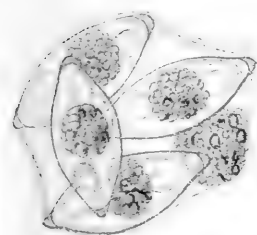
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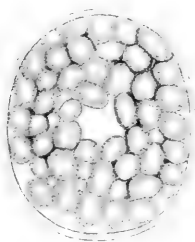
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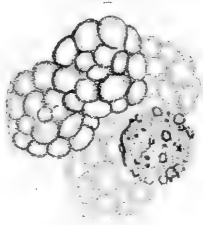
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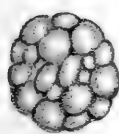
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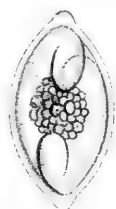
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Chromidia and the Binuclearity Hypotheses: A Review and a Criticism.

By

C. Clifford Dobell,

Fellow of Trinity College, Cambridge: Balfour Student in the
University.

With 25 Text-figures.

SINCE the seed of the chromidia hypothesis was sown by Richard Hertwig in 1902, it has displayed such an amazing ability to absorb new or previously uncorrelated facts, for its own growth, that it now—in its more mature form—stands out as one of the most conspicuous objects in the whole wide field of cytology. And it has not—one may be allowed to think—merely flourished on the soil where none other could take root: it has also, in so doing, thrown into the shade many a less showy upgrowth. Yet it is not beyond the bounds of possibility that these smaller growths, being rooted in a firmer foundation of facts, may remain to ripen long after the chromidia hypothesis has fallen to the earth—from the sheer weight of its own overgrowth and the insecurity of the ground in which it grew.

The chromidia hypothesis took origin in protozoology. But it has since pushed out its roots so far that they now extend and ramify in other domains of zoology, and bacteriology. The result is that it is very difficult to view in its entirety.

A most important offshoot from the original conception of chromidia has been a hypothesis of the binuclear nature of

the cell—a hypothesis which has been most ably advocated by Goldschmidt. This hypothesis of binuclearity,¹ as I shall call it, does not stand alone. There is at least one rival hypothesis which also seeks to demonstrate the double nature of the cell nucleus.

Now to comprehend the chromidial hypothesis and its closely-connected conceptions of binuclearity² it is necessary to be familiar with a very large part of the modern literature of protistology, and also with much cytological research in general; because the branches of the chromidia hypothesis have become twisted and tangled among the branches of the neighbouring binuclearity hypothesis—so much so, in fact, that it is nearly impossible to find out where one ends and another begins. The only sure way is to trace the offshoots from the parent stem.

It will be my aim in this essay to set out briefly and baldly all the main facts regarding chromidia, and to make such deductions as seem justifiable; afterwards, to discuss the hypotheses based on these facts; and finally—as this will involve a discussion of one binuclearity hypothesis—to criticise the other binuclearity hypotheses which are at present often confused with the idea of chromidia. To this end I have endeavoured to discover and verify facts wherever possible for myself. But my main source of information has naturally been the immense cytological literature which has grown up in the last few years. From its very size it would, of course, be quite impossible to enter into details in a short space. But I shall try, by selecting the most important points, to place the essential facts side by side in such a way that the value of the hypotheses arising from them will become evident. I wish to show that prevailing opinions are

¹ I have used the word “binuclearity” as an English translation of the various expressions commonly used in Germany, e.g. “Doppelkernigkeit,” “Kerndualismus,” “Kernduplizität,” “Kerndimorphismus,” “Binuklearität.”

² Already these hypotheses are occasionally honoured with the name of “theory”—and latterly even “law”!

not too firmly founded, and that a critical review of the facts does not justify all the inferences which have been drawn from them.

My object therefore is to discuss first the facts, secondly the speculations based upon them; endeavouring, by selecting the essential, to sacrifice detail for the sake of brevity.

TERMINOLOGY.

Before going any further I must define my terms. I shall use throughout only the two names introduced by Hertwig ('02), namely, chromidia and chromidial net (*Chromidien*, *Chromidialnetz*). Other terms are superfluous. By chromidia I understand any fragments of chromatin—irrespective of their shape or function—which lie freely in a cell,¹ without being massed together into a definite nucleus.² By chromidial net I understand any netlike arrangement of chromatin lying freely in the cytoplasm—regardless of its function. Both terms are purely morphological. It is sometimes convenient to speak of a whole system of chromidia—considered as a unit—in the singular number, as a chromidium.

Of other terms which have been used the following are the most important. Goldschmidt ('04a) employs the terms chromidia in the wider sense, for all chromidial structures of unknown function; chromidia (*sensu stricto*) for chromidia taking part in the vegetative functions of the cell; sporetia for chromidia which take part in forming gametes. This nomenclature has a physiological basis, and is difficult to use—except in a very few cases—owing to our present ignorance. Goldschmidt also introduced the term chromidial apparatus for any system of chromidia.

Mesnil ('05) uses a terminology which also has a physio-

¹ In the widest sense of the term.

² With Schaudinn I believe the nucleus should be defined morphologically. The above definition is not intended to embrace chromatin particles of extraneous origin (e. g. ingested bodies).

logical foundation; chromidia, used generally, like Goldschmidt's "chromidia in the wider sense"; trophochromidia, for chromidial structures of a vegetative function; idiochromidia, for chromidia which enter into the formation of gametes.¹

Schaudinn's ('05) three parallel terms are chromidia, somato-chromidia, gameto-chromidia. Other writers use various paraphrases of these, such as "somatic chromidia," "trophic chromidia," "vegetative chromidia"; and "gametic chromidia," "generative chromidia," "propagative chromidia," etc.

I will mention only one more term, used by Calkins ('05)—protogonoplasm. This unwieldy word is used to designate chromidia taking part in gamete formation. The self-explanatory term "distributed nucleus" is also used by this writer, though similar expressions (e. g. "diffuse nucleus") have long been in use.

I.

I will now endeavour to summarise the state of our knowledge regarding the existence of chromidia and their probable function in the Protista (Protozoa and Bacteria) and Metazoa. My aim here is to give facts, and to steer clear of hypothesis for the present.

(A) CHROMIDIA IN PROTOZOA.

(1) I will begin with the Heliozoa, as the chromidia hypotheses largely took root in this group. I refer, of course, to the magnificent researches of R. Hertwig on *Actinosphaerium*. From the immense mass of detail discovered by Hertwig and his school I select the following facts:

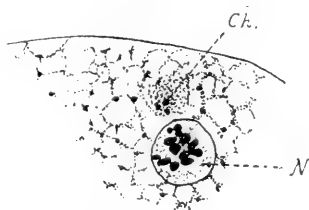
Hertwig ('99a) gave the first description of chromidia in

¹ Cf. Lubosch's ('02) terms, "trophochromatin" and "idiochromatin."

Actinosphærium (text-fig. 1). They are in the form of chromatin strands or granules lying in the cytoplasm, and are formed from the nuclei. Their formation may be induced either by over-feeding or by starving the animal. They are simply metabolic products—explicable, perhaps, by Hertwig's "Kernplasmarelationtheorie" (cf. Hertwig, '03, etc.). Hertwig named them "chromidia" in 1902. He further found that, during degeneration, the nuclei of *Actinosphærium* became enormously enlarged and hyperchromatic, and finally underwent fragmentation into chromidia (Hertwig, '00, '04; Howard, '08). These are the essentials.¹

(2) Let us pass on to the *Thalamophora*. Hertwig ('87)

TEXT-FIG. 1.



A portion of an *Actinosphærium* in a chromidial condition. *N.* nucleus; *Ch.* chromidia, formed from the nuclear chromatin. (The entire cytoplasm is filled with chromatin fragments lying in the walls of the alveoli.) (After R. Hertwig, '04.)

noted in *Arcella* an arrangement of extra-nuclear chromatin similar to that which he had already recorded in *Radiolaria* (vide infra). He described a "nuclear band" in addition to the vegetative nuclei.

Chromidia were discovered in *Polystomella* by Lister ('94, '95), but he was unable to decide upon their significance. Rhumbler ('94) probably observed chromidia in *Saccamina*, but was likewise unable to interpret their meaning. The chromidia in *Polystomella* were also seen by Schaudinn ('95a).

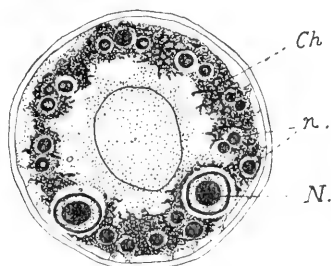
In 1899 Hertwig succeeded in fully tracing the develop-

¹ Similar processes occur in *Actinophrys* also (Distaso, '08).

ment of secondary nuclei from the chromidial mass—or, as he then called it, “the extra-nuclear chromatin net” of *Arcella* (text-fig. 2). And it has since been shown by Elpatiewsky ('07) that the macro- and micro-amœbæ, into whose formation the secondary nuclei enter, are gametes which conjugate in pairs.¹

When Hertwig ('02) introduced the name “chromidial net” for this extra-nuclear chromatin in *Thalamophora* its real meaning was still quite obscure. The riddle was solved by Schaudinn ('03). He found that the chromidial net (in *Polystomella*, *Centropyxis*, and *Chlamydophrys*) is a mass of chromatin—probably derived in the first instance

TEXT-FIG. 2.



Arcella vulgaris. *N.* primary nucleus; *Ch.* chromidium (extra-nuclear chromatin), in which the secondary nuclei (*n.*) are forming. (After R. Hertwig, '99.)

from the nucleus—which finally gives rise to the nuclei of minute gametes, which conjugate in pairs.

Other workers have extended Schaudinn's observations. In *Diffugia* (Zülzer, '04; Awerinzew, '06) the chromidia give origin to secondary nuclei,² which later enter into the

¹ Since this paper was written the interesting work of Swarczewsky ('08) on *Arcella* has appeared. In addition to confirming previous observations, this observer has found that a kind of conjugation (“chromidiogamy”) may take place between the entire chromidial masses of two individuals. A phenomenon to some extent parallel occurs in the giant disporic Bacteria, *B. bütschlii* (Schaudinn, '02) and *B. flexilis* (Dobell, '08a).

² And also form glycogen (Zülzer).

composition of gametes. A similar condition appears to prevail in *Euglypha*, *Trinema*, *Hyalosphenia*, *Nebela*, etc. (Awerinzew, '06).

Schaudinn's observations on *Polystomella* have been largely confirmed also in the case of *Peneroplis* (Winter, '07). Lister ('06) has already given a brief review of the nuclear phenomena in the Foraminifera.

Recently Doflein ('07) has re-examined many *Thalamophora*—namely, *Arcella* (2 species), *Platoom*, *Euglypha* (2 sp.), *Trinema*, *Gromiella*, *Lecquereusia*, *Nebela* (2 sp.), *Diffugia* (5 sp.), *Pseudodiffugia*, *Centropyxis*, *Cochliopodium*. A chromidial net was found in all, though its nuclear origin was not clearly made out. Its form shows great variation, being sometimes compact, sometimes diffuse. And it also varies considerably as regards the relative quantities of plastin and chromatin present in it. On the whole it seems that the chromidial net of the *Thalamophora* is a structure of nuclear origin whose chief purpose is to supply gamete nuclei.

(3) *Amœbina*.—Amongst the amœbæ three forms have received special attention—*Entamœba coli*, *Peloxyma*, *Amœba proteus*.

In the first, *Entamœba coli* Loesch, Schaudinn ('03) found that an autogamy takes place, in which chromidia play a part. Two daughter-nuclei in an encysted animal break up into chromidia, which are subsequently, in part, eliminated. The remaining chromidia mass themselves together to form two new nuclei, which, after each giving off two "polar bodies," become progamete nuclei. Each then divides, giving two gamete nuclei, which fuse in opposite pairs, to form two zygote nuclei.

It is unfortunate that the recent confirmation of much of this remarkable work by Wenyon ('07), in *E. muris*, has failed to corroborate the details of the history of the chromidia.

Entamœba histolytica (Schaudinn, '03) appears to have a chromidial net like that seen in the *Thalamophora*.

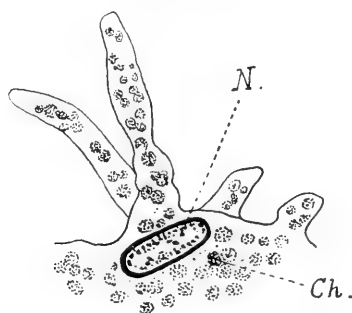
Chromidia were first found in *Pelomyxa* by Goldschmidt ('05). His discovery was confirmed by Bott ('06), who agreed that they were products of the nucleus, like those of *Actinosphærium*. They are produced when the animal hungers. But Bott was able to show further that chromidia play an important rôle in sexual reproduction. All the nuclei fragment, forming "somato-generative chromidia," of which a part degenerates and is cast out. The rest increase in size and form new nuclei, which—after eliminating more chromatin in the form of chromidia, and undergoing certain changes—give rise to gamete nuclei. Thus, in its essential points, gametogenesis in this creature resembles that of *Entamœba coli*.

Chromidia have been described in *Amœba proteus* by Calkins ('05). The nucleus was said to divide by mitosis,¹ until, after repeated division, a multinucleate condition of the cell resulted. These "primary nuclei" then broke up into "secondary nuclei" (by chromidia formation), and the "secondary nuclei" divided to form the hypothetical gamete nuclei. Since publishing this description Calkins has re-investigated the same material upon which these "evidences of a sexual cycle" were based. He now (Calkins, '07) comes to a quite different interpretation, and claims to have discovered the "fertilisation" of *Amœba*. The "secondary nuclei" are now said not to divide, but to fuse in pairs—thus undergoing a kind of autogamy. I do not wish to enter into a long discussion of this matter, but I must point out—as the fate of the chromidia bears upon the present subject—that Calkins' account is, by his own showing, impossible to accept. Apart from the fact that the whole story is based upon only a few preserved specimens, there are serious discrepancies in

¹ The "mitosis," as far as one can judge from Calkins' figures, is quite unlike mitosis as usually understood. Awerinzew, moreover, has described and figured in detail the mitosis of this organism. Judging from my own impressions and from Awerinzew's description, I am inclined to believe that Calkins' figures do not represent division stages at all.

his two accounts. When he now desires to show that the secondary nuclei fuse and do not divide, he adduces as evidence—inter alia—the statements that “if the nuclei were dividing we should find dumb-bell shaped figures with the diameter of the nuclei drawn out at right angles to the plane of division. This is not the case. . . . We should expect to find connecting strands of chromatin substance between the recently divided karyosomes . . . but no such connecting strands exist. . . . We should expect to find the daughter-karyosomes elongated in the axis at right angles to the plane of division. . . . Such is not the case.” How

TEXT-FIG. 3.



Part of an *Amoeba proteus*, containing “chromidia” (gametes of *Allogromia*). *N.* nucleus; *Ch.* “chromidia.” (After Prandtl, '07.)

are we to accept such statements, when, to prove that the nuclei were dividing, he originally not only described but figured all these stages of which he now denies the existence? (See Calkins, '05, Pl. 3, fig. 23.) So sure was he of this division that he even called it “a modified mitosis,” and described the karyosome as a division centre, like the nucleolo-centrosome of *Euglena* (text-fig. 25).

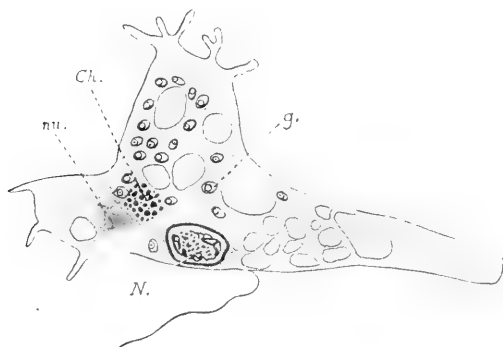
As Prandtl ('07a) has pointed out, Calkins' “gametes” of *Amoeba* are probably the gametes of parasites allied to *Allogromia*, whose remarkable life-history Prandtl carefully worked out. I cannot at all agree with Calkins in saying that if his secondary nuclei “are parasites, then the secondary

nuclei of *Arcella*, *Polystomella* and *Entamoeba* must likewise be parasites." Nor even from his description can I regard the "fertilisation" of *Amoeba proteus* as "strikingly similar to that of *Entamoeba coli*." The sexual phase—if it exist—in *Amoeba proteus* remains still unknown.

The facts about "chromidia" in *Amoeba* are therefore much too doubtful to allow of any profitable discussion at present.¹

(4) *Rhizomastigina*.—In the mastigamœbæ (*Masti-*

TEXT-FIG. 4.



Mastigella vitrea Goldschmidt, (a mastigamœba). *N.* nucleus; *Ch.* chromidia; *nu.* nucleolar substance; *g.* fully-formed gamete. (Modified from Goldschmidt, '07.)

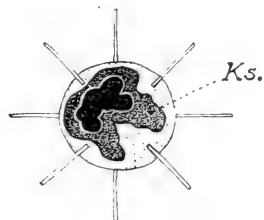
gella and *Mastigina*) we have one of the most carefully described cases of chromidia formation (Goldschmidt, '07). Chromidia—consisting of both "nucleolar substance" and chromatin—are extruded from the nucleus. In the cytoplasm they become aggregated at certain points and form gamete nuclei (text-fig. 4). The main nucleus remains behind, for a greater or less period, but in the end perishes.

(5) *Radiolaria*.—A structure like the chromidial net of *Thalamophora* was long ago described in *Acanthometrids* by

¹ Chromidia are described in *A. diploidea* (Hartmann and Nägler, '08) and some other species, but their significance seems to me to be very questionable.

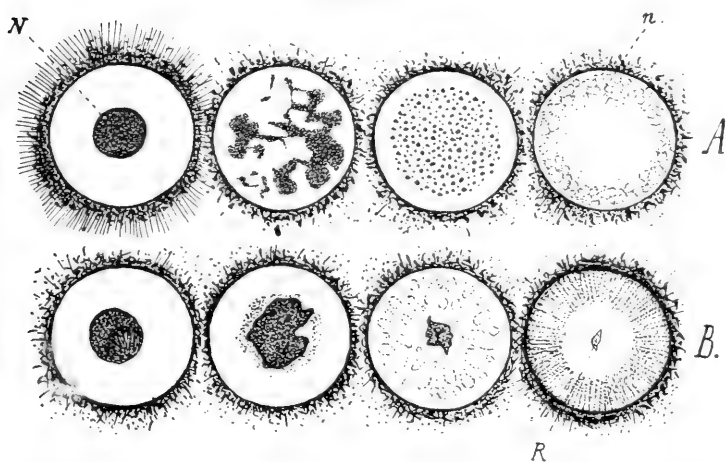
Hertwig ('79) as a "Kernrindenschicht" (text-fig. 5). Secondary nuclei (? gamete nuclei in all probability) are differen-

TEXT-FIG. 5.



A radiolarian, *Acanthochiasma krohnii*, showing the remarkable cortical layer ("Kernrindenschicht," *Ks.*) of the nucleus. This is probably the homologue of the chromidium of Thalamophora. (After R. Hertwig, '79.)

TEXT-FIG. 6.



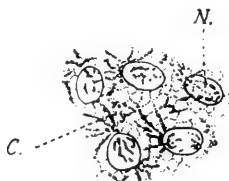
Chromidia in a radiolarian—*Thalassicolla*. *A*, formation of isospores; *B*, of anisospores (probably gametes). In both cases the primary nucleus (*N.*) breaks up into chromidia, which give rise to secondary nuclei (*n.*) entering into the formation of the swarm-spores. In the formation of anisospores, a part of the nucleus remains behind (*R.*). The drawings are of the central capsule of the organism. (From Brandt, modified.)

tiated from it in subsequent development, just as in *Arcella*, etc. (Hertwig, Porta).

The formation of zoospores in Radiolaria was described by Hertwig, but in more detail by Brandt, whose results have become fully known during only the last few years ('02, '05). It appears from his researches (e.g. in *Thalassicolla*) that the entire nucleus fragments into chromidia, which later form the nuclei of isospores (asexual reproduction). But in the formation of anisospores (probably gametes) only a part of the nuclear material goes into chromidia, which subsequently form the nuclei of the swimmers. The nucleolus stays behind and perishes with the remains of the parent organism (cf. *mastigamœbæ*). (Text-fig. 6.)

This account has received confirmation from the work of

TEXT-FIG. 7.



Part of a plasmodium of *Plasmodiophora brassicæ*.
N. nucleus; C. chromidia. (After Prowazek, '05.)

Schouteden ('07), who was the first to bring these phenomena into line with the other work on chromidia.

(6) Mycetozoa.—The chief work on chromidia in this group has been done by Prowazek ('04a, '05). He has found that the nuclei in the plasmodium of *Plasmodiophora* at one period in their development give up chromatin—in the form of chromidia—into the cytoplasm, and then after undergoing further changes give rise to gamete nuclei (text-fig. 7). Conjugation takes place as the spores are formed. Chromidia therefore take part in the vegetative existence of the organism. The sexual process in other Mycetozoa is not very well known. But recent work (Pinoy, '08) has shown that in one case at least (*Didymium*) there exist sexually differentiated plasmodia from the first.

(7) Mastigophora.—Chromidia have been described in

several flagellates. Prowazek ('03) recorded the presence of a "chromidium" in *Bicosæca*. He subsequently ('04) found a similar body in *Bodo lacertæ*. This structure lies near the nucleus, but it is difficult to see why it is called a "chromidium." Of its origin and fate nothing is known. It stains (in *Bodo*) with iron-haematoxylin but not with other chromatin stains, and perhaps consists of plastin¹ (text-fig. 8).

Prowazek has further described ('04) the formation of "chromidia" as a preliminary to a remarkable process of autogamy in *Bodo*. I will not discuss this further here as I have gone into the matter more fully elsewhere (Dobell, '08c). Suffice it to say that Prowazek probably mistook stages in

TEXT-FIG. 8.



Bodo lacertæ, from a preparation stained with hæmatoxylin and eosin. The so-called "chromidium" (*ch.*) is stained bright red, in striking contrast with the violet nucleus (*n.*). (Original.)

the development of yeast-like organisms for stages in the life-history of *Bodo*. The "chromidia" are reserve material. At all events the existence of chromidia in this animal is very doubtful.

Chromidia are said to play a part in the life-history of *Hæmoproteus* (*Trypanosoma*) *noctuæ*, (Schaudinn, '04, '05). They appear to be of a metabolic nature, as in *Actinosphærium* (cf. pp. 282, 283).

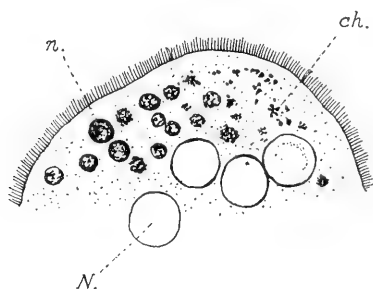
There are some other cases of chromidia recorded in flagellates, but they are not very satisfactory. In *Joenia*

¹ In Rhizopods the chromidial net may consist largely of plastin, and contain very little chromatin, so possibly this structure in *Bodo* is of a similar nature. Cf. Doflein ('07): "In *Trinema* conditions occur in which the chromidial body fills the apical part of the delicate shell as an almost compact, uniform mass of plastin."

(Grassi and Foà, '04), chromidia are described in the ordinary vegetative animal, but no particulars of their origin or function have been given. Perhaps they are really food bodies. Calkins ('98) has described the nucleus of *Tetramitus* as having its chromatin scattered through the cytoplasm during resting stages. This has never been confirmed, and I think it quite possible that the "chromidia" are here also merely ingested food masses, which often stain very strongly in such flagellates.

Awerinzew ('07) says that a part of the chromatin—in the

TEXT-FIG. 9.



Opalina: part of an individual which is preparing to form gametes. *N.* primary nucleus, which has given up most of its chromatin as chromidia (*ch.*). The latter, by aggregation at various points, give rise to the secondary nuclei (*n.*). (Modified from Neresheimer, '07.)

resting animal—is in the form of chromidia in *Chilomonas*. Prowazek ('07a) contests this, and believes Awerinzew's specimens were badly fixed. He himself ('03) found no chromidia in this animal.

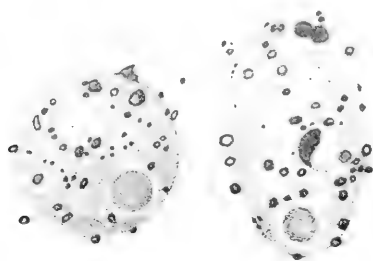
Quite recently Swellengrebel ('08) has found granules of "volutine" (A. Meyer) in *Trypanosoma*. He says: "It is evident these granules of volutine, from their nuclear origin, ought to be considered as chromidia." With this I cannot agree. They are not chromatin, therefore to my mind they are not chromidia.

On the whole the chromidia of flagellates are at present of

too doubtful a nature to allow of any profitable discussion regarding them.

(8) Ciliata.—The best instance of chromidia playing a part in the life-cycle of a ciliate is to be seen in *Opalina*, (Neresheimer, '07) (text-fig. 9). At a certain period in its development *Opalina* extrudes chromidia from its nuclei into the cytoplasm. The chromidia then collect themselves at various points, and so build up new nuclei—the original nuclei perishing. These secondary nuclei, after undergoing a chromatin reduction, become the nuclei of gametes. The history of the chromidia in this animal is therefore rather like a multiple version of that in *Thalamophora*.

TEXT-FIG. 10.



Degenerating fragments of *Opalina*, with nuclei in a chromidial condition. (The large bodies surrounded by a pale area are "eosinophil" bodies.) (After Dobell, '07a.)

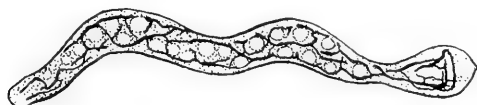
Chromidia are also formed in *Opalina*—as in many other Protozoa—during degeneration (Dobell, '07a) (text-fig. 10).

Gonder ('05) has given a description of remarkable chromidial phenomena in *Opalinopsis* and *Chromidina*. I have re-investigated these forms (Dobell, '08d) and arrived at a very different conclusion from Gonder's. There is no complicated series of chromidial changes in *Opalinopsis* during division. The nucleus is in the form of a network ("chromidial net" if one likes to call it so, though there is no evidence that it is in any way homologous with the chromidial net of *Thalamophora*), and remains so during division. In *Chromidina* the nucleus is also in the form of

a net (text-fig. 11). The "chromidia" in these two forms are in part ingested food material and in part appearances due to imperfect fixation—artifacts. As I have already discussed the matter elsewhere I will say no more about it here.

The only other case of chromidia which need be considered in this group is that of *Cryptochilum*. It is stated by Russo and Di Mauro ('05a) that there is a chromidial net in the posterior region of this holotrichous infusorian. But they have also described ('05) the fragmentation and digestion of the macro-nucleus in the same region. Is the "chromidium" merely the degenerated and broken-up macronucleus? It is impossible to say from their account. Further, they have described ('05b) the conjugation of this animal, but without

TEXT-FIG. 11.



Chromidina elegans, an infusorian having its nuclear apparatus in the form of a network. (Original.)

enlightening us as to the rôle of the chromidium—which is neither mentioned nor figured. It may be that it is either a worn-out remnant of the macronucleus, or possibly a mass of ingested food bodies. It is useless to attempt to argue about it before we have more definite data.

(9) Sporozoa.—There are some good examples of chromidia formation in this class of Protozoa. I select the following. In *Eimeria schubergi* (Schaudinn, '00) the nucleus of the micro-gametocyte undergoes an analysis into chromidia, which become aggregated at various points at the periphery of the organism and so synthesise the chromatin microgametes. A similar process takes place in *Adelea* (Dobell, '07) (text-fig. 12), but here a chromidial network is formed. In this form also, formation of macromerozoites from a macroschizont is accompanied by a series of nuclear changes analogous to those just noticed in *E. schubergi* (Siedlecki, '99, Dobell, 07).

The formation of secondary nuclei from chromidia has been described in *Lymphocystis* (see Awerinzew, '08). The same kind of nuclear phenomenon has, in addition, been described by Siedlecki ('98) in *Aggregata* (*Klossia*, *Eucoccidium*, etc.), during the formation of sporoblasts and microgametes. Recently this has been challenged by Moroff ('08), who has described most remarkable chromidial formations, centrosomes, etc., and based a number of speculations thereupon. I have been engaged in studying these parasites for some time past, and hope to be able to consider Moroff's work in detail later. For the present I will merely

TEXT-FIG. 12.



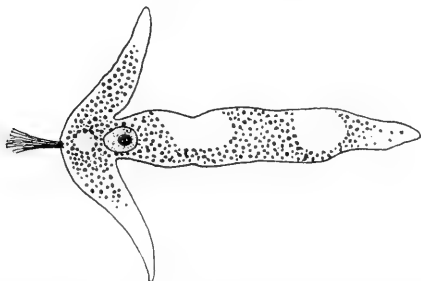
Formation of microgametes in *Adelea ovata*. (After Dobell, '07.)

say that, in most respects, my work so far confirms and amplifies that of Siedlecki. Moroff's "chromidia," etc., are to my mind in great part artifacts, due to defective cytological methods.

The Gregarines furnish many examples of chromidia. Chromatin particles in the cytoplasm have been noticed by many observers, in many different species, for a long time past. They vary greatly in amount. A very good instance has been described and figured by Cecconi ('03) in *Anchorina*, but he was unable to discover their origin or significance (text-fig. 13).

According to Drzewiecki ('03) most remarkable nuclear phenomena occur in *Monocystis*. In the vegetative period of development the nucleus is said to undergo complete fragmentation into chromidia. A new nucleus is then gradually

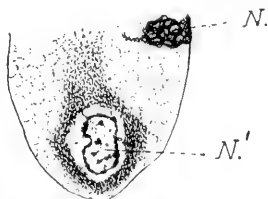
TEXT-FIG. 13.



Anchorina sagittata, a gregarine. The protoplasm is filled with "chromatophile granules" (chromidia). (After Cecconi, '05.)

built up from new chromidia, which make their appearance in the cytoplasm—the first-formed chromidia disappearing (text-fig. 14). Drzewiecki ('07) has lately described a similar phenomenon in *Stomatophora*, introducing new terms into his

TEXT-FIG. 14.



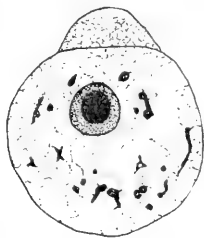
Posterior end of a gregarine, *Stomatophora coronata*. The original nucleus (*N.*) has broken up, and a new nucleus (*N'*.) is in process of formation from chromidia in the cytoplasm (?). (After Drzewiecki, '07.)

description ("nucleolids," "chromatogens," etc.). His account is based entirely on the study of fixed and stained specimens—in the second paper, on the study of a single preparation stained by Heidenhain's method! The results have been regarded with some scepticism already (e. g. by

Lühe, '04), and I think it is almost certain, from the recent work of Kuschakewitsch ('07), that Drzewiecki has arrived at his results by combining a series of degeneration phenomena. At all events, Drzewiecki's account stands in need of confirmation, and cannot be accepted at present.

It appeared from the work of Léger ('04) and others, that the chromidia of gregarines were probably the same sort of thing as those of *Actinosphærium*. But the most careful recent work—that of Comes ('07)—has put the matter in a different light. Comes studied *Stylorhynchus* and *Stenophora* (text-fig. 15). He observed the chromidial changes

TEXT-FIG. 15.



A small *Stenophora juli*, showing deeply stained particles (chromidia) in the cytoplasm. (From a borax-carminé preparation. [Original].)

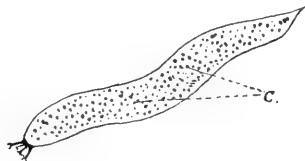
which occurred with change of nutrition, temperature and season. The important fact brought out by this study is that the chromidia are not of nuclear origin—they are metabolic products in the cytoplasm. Their part is played in the vegetative life of the organism. In view of these facts it is obvious that the chromidia of gregarines require cautious consideration in relation to the nucleus.

Before passing to the bacteria, I may here note the nuclear apparatus of a very remarkable, and as yet unclassifiable, organism—*Siedleckia nematoides* (Caullery and Mesnil, '98, '99). I have lately studied this parasite, from a new host, *Aricia fœtida*. *Siedleckia* contains small chromatin

masses, whose number varies according to the size of the animal, and which multiply by a simple division (text-fig. 16). They cannot properly be called nuclei. They should be regarded, I think, as composing a nuclear apparatus consisting of scattered fragments of chromatin—a chromidial system—as in some bacteria (e. g. *B. flexilis*, Dobell, '08a). In large animals they are present in immense numbers, but at no period do they—individually—possess the attributes of a formed nucleus.

In some Protozoa nuclear reduction by chromidia formation takes place in a gamete preparatory to conjugation (e. g. macrogametocyte of *Adele* (Siedlecki, '99), and in

TEXT-FIG. 16.



Large *Siedleckia nematoides* (from *Aricia fœtida*).
c. chromatin fragments in the cytoplasm. (Original.)

Monas (Prowazek, '03). Their meaning is bound up with the general problem of nuclear reduction, and I shall say no more about it here.

(B) CHROMIDIA IN BACTERIA.

In spite of the great discussion which has raged—and still rages—round the problem of the bacterial nucleus, there is a large and growing body of evidence to show that some, at least, of the granular inclusions in bacteria consist of chromatin (cf. Guilliermond, '07). In part, however, the granules ("metachromic granules," "red granules," "volutine granules," etc.) probably consist of some reserve material (cf. Guilliermond, '06, '07). It can hence be said that certain bacteria ¹

¹ And probably also Cyanophyceæ.

have their chromatin in a chromidial condition. (Cf. also the morphology of *Achromatium*, as carefully studied by Schewiakoff, '93.)

In large bacteria which have been carefully studied, the chromidia are seen to come together to form a nucleus-like body during spore formation (cf. Schaudinn '02, '03a; Dobell, '08; Guilliermond, '08, etc.) (text-fig. 17).

It appears equally certain, however, that some bacteria—or organisms at present classified as such—possess a well-differentiated nucleus, and not chromidia (Vejdovský, Mencl, etc.). The nucleus may sometimes be in the form of a filament or otherwise modified.

So much for the true bacteria. We may here consider, as an appendix to them, that interesting little group of protists,

TEXT-FIG. 17.



Bacillus flexilis. The nuclear apparatus is seen to consist of chromatin particles scattered through the cytoplasm. (Original.)

the spirochæts. In some, at least, of these the chromatin appears to be arranged, wholly or in part, in the form of chromidia. I will give *Spirochæta plicatilis* as an instance. In this organism, "The nuclear apparatus consists of a thread-like structure running in the long axis . . . whilst the vegetative nuclear mass surrounds this thread in the form of granular chromidia" (Schaudinn, '05a).

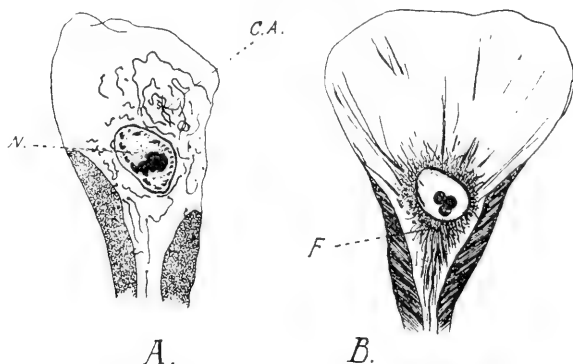
(c) CHROMIDIA IN METAZOA.

Descriptions of free chromatin particles in metazoan cells—homologized with the chromidia of the Protista—are not few. The two most important cases—the two which I shall chiefly discuss here—are the chromidia of the tissue-cells of nematodes, and the chromidia in the gametogenesis of gastropods. These are the mainstays of the arguments, in favour of the chromidia hypotheses, derived from multicellular organisms.

The Chromidia of Nematodes.—Goldschmidt ('04, '04a), has described at considerable length certain curious chromatin strands, which occur in various tissue-cells—especially muscle-cells—of *Ascaris*. These structures he calls the chromidial apparatus of the cell. Upon them Goldschmidt's binuclearity speculations are largely founded.

The chromidial apparatus is said to consist of chromatin extruded from the nucleus when the cell is in a state of activity—the amount of chromatin being an index of the

TEXT-FIG. 18.



A. A muscle-cell of *Ascaris lumbricoides*, after one hour's tetanus, showing the "chromidial apparatus" (C.A.), which is supposed to have come from the nucleus (N.). (In the original figure—from a hæmatoxylin preparation—the nucleus is coloured violet, the "chromidia" black.) (After Goldschmidt, '05.)

B. A muscle-cell of *Ascaris ensicaudata*, showing the supporting framework (F.) in the cytoplasm. (After Vejdovský '07.)

Both figures are from transverse sections, so that only a part of the cytoplasmic structures is seen.

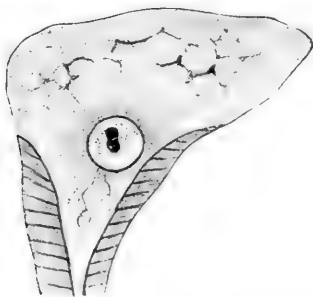
degree of activity of the cell. Thus, when an *Ascaris*¹ is stimulated to violent muscular movement, the chromidial apparatus is found more strongly developed in the cell (text-fig. 18).

Leaving out of the question for the moment the vast edifice of speculation which Goldschmidt has erected on these obser-

¹ *A. lumbricoides* and *A. megalocephala* were used.

uations, we must inquire, "What is this chromidial apparatus?" The evidence that it is chromatin from the nucleus is not—to me—convincing, but it has been widely accepted. The most important evidence yet brought forward in opposition to Goldschmidt is that of Vejdovský ('07). This investigator—and his opinion is of special weight, owing to his long experience in matters of vermian cytology—has examined another species of *Ascaris* (*A. ensicaudata*) with this result. He finds¹ remarkable fibrillar structures, which "must be regarded as only a supporting framework" of the cell. He believes that Goldschmidt's "chromidia" are merely broken

TEXT-FIG. 19.



Muscle-cell of *Ascaris lumbricoides*, showing structure of cytoplasm in a fixed and stained cell. (Original.)

up parts of this fibrillar system—in reality artifacts due to the methods employed. (Cf. fig. 18.) As he himself concisely expresses it, "The chromidial apparatus described by Goldschmidt represents the strands of the 'normal' fibrillar framework—much damaged and torn as a result of the violent action of the reagents employed—which is probably derived from the original ray-system of the centropiasm." (Vejdovský, '07, p. 89, and cf. Fig. 19.) With regard to the staining reactions of these fibrils, Vejdovský further adds that the strands of the "primary centropiasm" in *Fridericia* also

¹ These supporting fibrils have been long known to cytologists—including, of course, Goldschmidt.

stain (with iron-hæmatoxylin or brasilin) just like the nuclear chromatin.

The increase of chromidia with increased activity is thus explained: the more prolonged and violent the stimulus, the greater the damaging and tearing of the fibrils, and hence the greater the number of "chromidia."

Whether Goldschmidt or Vejdovský ultimately prove to be correct, it is important to note for the present that the "chromidia" of *Ascaris* may be really nothing more than much modified derivatives of centropasmic rays (cf. p. 303).

The Chromidia in the Gametogenesis of Gastropods.—The advocate for chromidia in the development of gastropod¹ eggs and sperms is Popoff. According to him ('07) chromidia are formed in the spermatocytes and oocytes at certain stages of development (cf. text-fig. 20A). They are extruded from the nucleus as chromatin granules. Personally I am far from being convinced of the nuclear origin of the "chromidia," either by his figures or his description.

Now the "chromidia" are really nothing more than the "pseudochromosomes," "Nebenkern," etc., already long known from the work of Meves, Platner, Bolles Lee and others (cf. Meves, '00). But for Popoff, "the observations (i. e. Popoff's on *Helix*) . . . show that the structures described by various authors under the names mitochondria, pseudochromosomes, archoplasm, ergastoplasm, Nebenkern, idiozome (only in certain cases) and idiozome remains, are referable to different isolated stages of one and the same developmental series of the chromidia." He considers his work to be an "undoubted proof" of this.

As a great deal has been written on this matter, I will content myself with citing the opinion of three other investigators of the same structures.

Murray ('98) found centrosomes in the Nebenkern of *Helix*. And he concluded that the Nebenkern was really the attraction sphere, and that in it "no structures exist in any way comparable to chromosomes." This conclusion was

¹ *Paludina* and *Helix*.

accepted by Boveri ('00), in whose laboratory the observations were made.

Ancel ('02) has given a most exhaustive account of the same structures. He believed that the pseudochromosomes and Nebenkern were stages in the development of the same thing, but that they were not formed from nuclear chromatin, being "the product of transformation of differentiated cytoplasmic filaments."

Bolles Lee ('02) says the Nebenkern in *Helix* is nothing but a degenerating bunch of spindle rays. He "can affirm that the Nebenkern is derived from the spindle with as much certainty as one can affirm that an oak is derived from an acorn."

In face of these assertions regarding the "chromidia" of *Helix* it is surely necessary for Popoff to bring some further proofs forward before we can accept his interpretation.¹

Attempts have been made to homologize various structures (mitochondria, etc.) in nerve-cells with chromidia, (e. g. by Goldschmidt, '04a; Popoff, '06, etc.) But the evidence is even less convincing than in the two cases already given. It seems not unlikely that they, like the "chromidia" of *Ascaris* and *Helix*, are really nothing more than the remains of centropasmic fibres. It is significant that this same result should have been arrived at in these different cases by quite independent observers.

Chromidia have been described in several other multicellular organisms, e. g. in dicyemids (Hartmann, '07). They are here said to play a part in the vegetative life of the animal, but the observations require confirmation. And this, indeed, may be said of most cases of chromidia in the Metazoa.²

¹ According to Wassilieff ('07) similar structures (mitochondria) in the spermatocytes of *Blatta germanica* originate from the nucleus, but are "no special kind of chromatin, but only superfluous chromatin."

² An interesting chromidial condition appears to occur also in sponges, e. g. in the gastral actinoblasts of *Clathrina cerebrum*, as described by Minchin ('98). I am indebted to Prof. Minchin for kindly calling my attention to the fact.

Now let us consider all these facts about chromidia, regardless of any hypotheses which have already been introduced to "explain" them.

First, it seems to me that the evidence at present is strongly in favour of the view that in the Metazoa most of the so-called chromidia are really scattered remnants of centropasmic fibrils or their derivatives—properly speaking, not nuclear chromatin at all. Consequently, I believe that any hypothesis which is based upon the assumption of their nuclear nature¹ has a very insecure foundation. But before we have more facts to go upon it seems to me premature to argue the matter further.

Secondly, I believe that certain facts regarding the Protista are sufficiently well established to permit of generalisations being made.

It is perfectly evident that under the name chromidia at least four quite distinctly different things are comprised, whose morphological resemblance alone allows of their sharing a common title. Physiologically they are quite different. First, chromidia may represent the normal condition of the chromatin in a vegetative cell which has no formed nucleus (e. g. in Bacteria, *Siedleckia*, etc.). Secondly, chromidia may be the products of cell metabolism—either of the nucleus (e. g. *Actinosphærium*)² or of the cytoplasm (e. g. *Stenophora*).³ Thirdly, chromidia may be decomposition products of the nucleus, due to degeneration or death of the cell (e. g. degenerating *Opalina*).⁴ And fourthly, chromidia may represent one stage in a process of multiple nuclear division (e. g. *Mastigella*).⁵ This process of nuclear division occurs frequently—though not exclusively (cf. isospores of *Radiolaria*, p. 289)—in the formation of gametes.

¹ I do not mean to imply that the centrosome and centropasm were not originally themselves derived from the nucleus. On the contrary, I regard this as highly probable.

² See p. 282.

³ See p. 297.

⁴ See p. 293.

⁵ See p. 288.

I will consider this last case in more detail, as it is the basis of much theorizing.

Whatever theoretical value we may give to the chromatin itself, it cannot be denied that chromidia represent an intermediate stage in the simultaneous formation of a number of nuclei from a single nucleus. The reason why we find this method of multiple division so frequently occurring in gametogenesis is, to my mind, quite obvious. It is an adaptation to ensure the formation of a number of gametes at the same time. From the very nature of the life-conditions of many Protozoa it is absolutely necessary for a large number of gametes to be formed at once; for a large number must usually, like the sperms of Metazoa, fail to fulfil their duty.

There are few accurate accounts of other methods of multiple nuclear division, but it has been studied carefully in at least one protozoon—*Calcituba* (Schaudinn, '95). Except for the fact that all the events take place inside the nuclear membrane, it is exactly comparable with the method by chromidia formation as seen in *Aggregata*, etc.

In *Thalamophora*, *Radiolaria* and *Rhizomastigina*, where the chromidium remains for some time as a permanent organella during the vegetative life of the cell, we see merely a device by which, through the independent growth of the chromidia, a larger brood of gametes can be eventually produced than by the sudden multiple division of a single nucleus.

The multiple nuclear division in *Opalina* is cloaked by the fact that the cell is originally multinucleate. This applies also to *Pelomyxa*. And here, apparently, nuclear reduction and multiple division occur at the same time, so that they obscure one another.

There is one other interesting conclusion which may be drawn from the facts regarding chromidia. It is that an actual cell death exists in the "immortal" Protozoa. Consider the following instances. In many of the rhizopods the primary nucleus and the remains of the cytoplasm are left

behind when the brood of gametes swims off to conjugate. The whole of this residuary mass then dies. The same fate overtakes the remains of the microgametocyte in coccidiids—e. g. *Adelea ovata*, *Eimeria schubergi*, etc. This does not indicate that the cell must be regarded as by nature containing two kinds of chromatin—somatic and generative—any more than it indicates that the cell by nature contains two sorts of cytoplasm. It simply shows us that a cell, or part of a cell, can get worn out with its life-activities and die. The residuum (*Restkörper*) is the corpse.

This same idea has already occurred to R. Hertwig ('06a), amongst others.

II.

And now to the hypotheses connected with chromidia. As Hertwig's original conceptions of chromidia began with *Actinosphaerium*, and have been woven into his hypothesis of the karyoplasmic relation,¹ I will begin with this.

The hypothesis states that "the relation of nucleus to protoplasm, the quotient $\frac{k}{p}$ —that is, the mass of nuclear substance divided by the mass of protoplasm—is a constant, whose magnitude is of fundamental importance for all vital processes influenced by the nucleus, for assimilation and organising activity, for growth and division." Now, if nucleus and cytoplasm do not grow at the same rate, the nucleus may become too large for the cell, a condition which may lead to degeneration and death. The nucleus, however, may reduce its size by giving up part of its chromatin—as chromidia—and so re-establish the normal relation $\frac{k}{p}$. The chromidia are thus a means for regulating the karyoplasmic relations.

The formation of chromidia by the microgametocyte of the

¹ I use this expression as an English equivalent of Hertwig's term, "*Kernplasmarelation*."

malaria parasite, in a recurrence of malaria—explained by Schaudinn ('02a) as a kind of sexual process—is also accounted for by Hertwig ('06) as a process which corrects the karyoplasmic relations.

The basis of this hypothesis is now so wide that it will be quite beyond the scope of this essay to discuss the large mass of literature relating to it. There are already many striking experimental facts in favour of the correctness of the hypothesis, and even if it is not destined to take its place as one of the fundamental theories of cytology it will have served as a working hypothesis of the very greatest importance.

The other hypothesis which sprang from the facts concerning chromidia is the hypothesis of binuclearity. It gradually took shape in the later work of Schaudinn, but has found its most ardent advocate in Goldschmidt ('04a, '05). From his work on nematodes (cf. p. 300), and a consideration of chromidia in the Protozoa, Goldschmidt came to the following conclusions:

“(1) Every animal cell is by nature¹ binucleate; it contains a somatic and a propagatory nucleus. The former presides over somatic functions, metabolism and movement . . . The propagatory nucleus contains especially the hereditary substances, which also possess the ability to generate a new somatic nucleus.

“(2) Both kinds of nucleus are usually united into a single nucleus—the amphinucleus. Separation may take place to a greater or less extent . . .

“(3) Complete separation of the two kinds of nucleus can be seen in only a few cases, in connection with reproduction in Protozoa and also in oogenesis and spermatogenesis of Metazoa.

“(4) In tissue cells the separation may be quite unnoticeable. . . . An almost complete separation may occur in ganglion and muscle cells. The somatic nucleus lies in the cytoplasm as the chromidial apparatus . . .

¹ “Ihrem Wesen nach.”

"(5) Cells with only a propagatory nucleus, but which can, of course regenerate a somatic, are found only in the gametes of protozoa, and in certain nutritive cells of the ovary—possibly also in many sorts of spermatozoa."

"(6) Cells with only a somatic nucleus are also possible: the residuum of gregarines, the reduced cells of *Ascaris*, certain muscle cells."

In the first place, it must be noted that the term "binuclearity" ("Doppelkernigkeit") is not a happy one. The conception is not of two nuclei but of two kinds of chromatin, and in this it differs from the other binuclearity hypothesis (Schaudinn-Prowazek-Hartmann, cf. p. 311). The idea would be more exactly expressed by a word such as "dichromaticity" ("Dichromatizität"). An actual somato-reproductive binuclearity exists only in such forms as the Infusoria, where somatic nucleus (meganucleus) and propagatory nucleus (micronucleus) are often completely separate. This arrangement—which, for Goldschmidt, shows a resolution of the nucleus into its primary parts—is, for me, merely a mark of the high degree of differentiation which the Infusoria exhibit in so many other ways besides. To my mind it is a specialisation, not a simplification. There is, moreover, some evidence to show that even here the two nuclei do not necessarily consist of two essentially different kinds of nuclear substance. For, as has been abundantly proved, the micronucleus can form a meganucleus after conjugation; and conversely, the meganucleus can probably form a micronucleus (Le Dantec, '97).

It is further to be noted that the "propagatory nucleus," far from being entirely concerned with reproduction, can in certain cases exhibit independent powers of metabolism and growth.¹ It is as unjustifiable to maintain that the micro-

¹ Cf. Mastigina, "The sporetia . . . must indeed nourish and reproduce themselves independently. And we must assume the same for the . . . chromidial net of the shelled rhizopods" (Goldschmidt, '07). And further, in *Arcella*: "The generative function (i. e. of the chromidial net) is indubitable. On the other hand, it is equally

nucleus (or its homologue the chromidial net¹) plays no part in the vegetative life of the protozoan cell, as it is to maintain that the germ cells of a metazoan individual play no part in its general somatic metabolism.

In that the nuclear conditions seen in Infusoria are specialised, and not primitive—to my mind—they show no more that the cell is by nature binucleate than a metazoan containing n different organs—each with its specialised nuclei—shows that the cell was originally by nature n -nucleate.

To say that the cell nucleus possesses the two distinct functions of growth and reproduction is a platitude. But to say that these two functions are restricted to special parts of the nuclear material is not warranted by the facts known to us at present. All the facts appear to me to point to the conclusion that growth and reproduction—somatic and propagative functions—are united in the same living nuclear molecule. One or other may come to preponderate, but that is the necessary result of cellular differentiation.

I think a great deal of error has crept into the chromidial hypothesis of binuclearity through the unfortunate application, originally, of a similar name to two quite different things—the chromidia of *Actinosphærium* (products of metabolism or disintegration) and the chromidial net of *Thalamophora* (a reproductive organ). When Goldschmidt added to these the chromidial apparatus of ascarids—again quite a different thing (cf. p. 302)—confusion was complete, and hence the deductions which at first sight appear so legitimate. To my mind, the facts by no means allow of the conclusions drawn by Goldschmidt (p. 307).

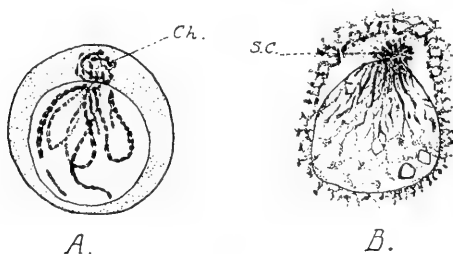
Starting from these—to me—false premises, Goldschmidt

certain that the chromidium fulfils trophic functions” (Elpatiewsky, '07). “That there exist pure gametochromidia, entirely without admixture of somatic nuclear matter, is improbable” (Schaudinn, '05).

¹ Cf. Swarczewsky, '08. From his work it appears that in *Arcella* the chromidial net gives rise to secondary nuclei, which enter not only into the gametes but also into asexual buds; so that here at least there is no justification for regarding the chromidium as purely gametic.

and Popoff ('07) have greatly extended the original chromidial hypothesis. For them, the "sphere" of *Noctiluca* (Ischikawa, Calkins, Doflein), and the "spongy

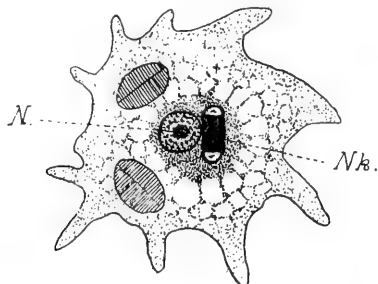
TEXT-FIG. 20.



- A. Formation of "chromidia" (*Ch.*) in the oocyte of *Paludina vivipara*. (After Popoff, '07.)
 B. Formation of the "spongy centrosome" (*S.C.*) from the nucleus in *Actinosphaerium*. (After R. Hertwig, '98.)

centrosome" of *Actinosphaerium* (Hertwig, '98), correspond to the "chromidia" of *Paludina* (cf. p. 302), all being chromidial structures. Further, the nucleolo-centrosome of

TEXT-FIG. 21.



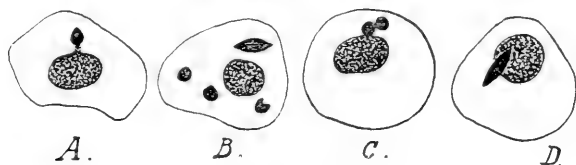
Paramoeba eilhardi. *N.* nucleus; *Nk.* Nebenkörper. The latter stains deeply with chromatin stains, and functions as a cytcentre. According to Goldschmidt and Popoff it represents a "chromidial apparatus." According to Hartmann and Prowazek, a kinetonucleus. (After Schaudinn, '96.)

Euglena (Keuten, '95) and the Nebenkern of *Paramoeba* (Schaudinn, '96) are each regarded by them as constituting a "chromidial apparatus" (text-figs. 21, 25).

Such speculations, to my mind, greatly exceed the limits of legitimate inference. Yet it has come to be the fashion of late to repeat that a binuclearity of this kind exists in all accurately-investigated Protozoa (e.g. Enriques, etc.).

One of the most striking pieces of evidence in favour of somato-generative binuclearity is seen in the life-history of the remarkable infusorian, *Ichthyophthirius* (Neresheimer, '08). The nucleus (amphinucleus) buds off a smaller nucleus, which divides into two. The latter then undergo two reduction divisions each, and finally fuse—thus enacting an autogamy. The zygote nucleus then re-enters the original nucleus and so reconstitutes a fresh amphinucleus (text-fig.

TEXT-FIG. 22.



Ichthyophthirius.

- A. The originally single nucleus gives off a micronucleus.
 - B. The micronucleus undergoes two divisions. Three of the four resulting nuclei degenerate—the fourth divides once more (spindle).
 - C. The spindle gives rise to a pair of nuclei which fuse (autogamy).
 - D. After fusion, the nuclei re-enter the original nucleus and fuse with it.
- (After Neresheimer, '08—schematic.)

22). It certainly appears as though we were here dealing with two different kinds of chromatin—trophic and gametic—united into one nucleus.

Entamoeba coli also seems to furnish strong evidence in favour of this view. In neither of these cases, however, is the evidence conclusive, and both stand in need of confirmation.

The second binuclearity hypothesis—which has, to a considerable extent, been confused with the one already discussed—is more properly so-called, for it has, as its basis,

the conception of an originally doubly nucleate cell. This hypothesis is much older than the chromidial idea, and is intimately bound up with speculations regarding the origin of the centrosome. I will try to sketch its history as briefly as possible, and then say something about its most recent developmental phase.

In 1891 Bütschli noticed a chromatin-staining centrosome in the diatom *Surirella*, and suggested that it might possibly be homologized with the micronucleus of an infusorian. A somewhat similar view was advanced by R. Hertwig ('92). He said that the ordinary nucleus of a metazoan cell might be regarded as a nucleus with little or no active substance, but rich in chromatin—the centrosome, however, as a nucleus which had lost its chromatin but retained its activity. This would thus presuppose the original cell to contain two nuclei.

Lauterborn ('93), continuing Bütschli's work on diatoms, also pursued the ideas which the latter had started. Before he had given a complete exposition of the result at which he had arrived, however, Heidenhain ('94) published an elaboration of Bütschli's original conception. He regarded the condition seen in the Infusoria—a cell containing two nuclei—as a primitive condition, and regarded the nucleus and centrosome of a metazoan cell as derived from the infusorian meganucleus and micronucleus respectively. As Lauterborn pointed out, this is in the highest degree improbable, as the arrangement seen in the Infusoria is a highly specialised one, and not primitive.

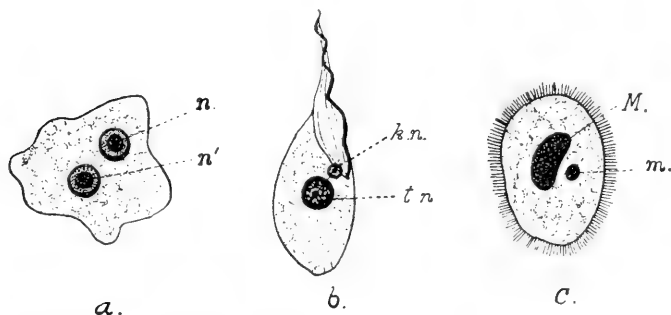
Lauterborn himself gave a full exposition of his views in 1896. As a starting point he takes, not the specialised binuclear condition seen in Infusoria, but a cell containing two exactly similar nuclei, *Amœba binucleata* Gruber (Schaudinn, '95b). From this primitive condition the meganucleus and micronucleus of Infusoria, and the nucleus and centrosome + central spindle of Metazoa, are supposed to have been collaterally evolved. Lauterborn supposes that in diatoms also the centrosome + central spindle represents one original nucleus, *Paramœba eilhardi* (Schaudinn, '96)

with its nucleus and Nebenkörper representing a stage intermediate between the diatom and the *A. binucleata* condition.

These views were all very clearly expressed and are the parents of the existing binuclearity hypothesis of Schaudinn and his followers.

Schaudinn's ('96a, '05, etc.) conception of binuclearity was chiefly based upon his observations on *Acanthocystis* and *Hæmoproteus* (*Trypanosoma*) *noctuæ*. In the latter we see an organism which is actually binucleate, there being a

TEXT-FIG. 23.



Illustrating three different kinds of binuclearity which actually exist in three different groups of Protozoa:—

a, in the Rhizopoda, *Amœba binucleata*, an organism with two exactly similar nuclei (*n. n'*).

b, in the Flagellata, *Hæmoproteus noctuæ*, which has two differentiated nuclei—kinetic (*k.n.*) and trophic (*t.n.*).

c, in an Infusorian. Here the nuclei are differentiated into a somatic (meganucleus, *M.*) and sexual (micronucleus, *m.*).

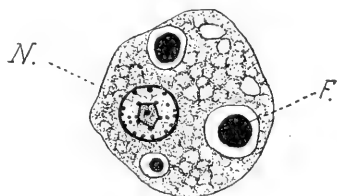
The three figures also serve to illustrate the starting points of the three binuclearity hypotheses,—namely those of Lauterborn, Schaudinn Hartmann and Prowazek, and Goldschmidt—respectively.

second nucleus (kinetonucleus) in addition to the main nucleus (trophonucleus). Both nuclei take part in conjugation, and at certain periods in the life-cycle they may be united into a single nucleus (synekaryon). The kinetonucleus (blepharoplast) is specially concerned with the locomotor functions of the cell.

Now it is this second nucleus—the kinetonucleus—which is

supposed to be the homologue of the metazoan centrosome. We thus have a conception of binuclearity which starts neither from the somato-gametic nuclear differentiation of infusoria (Goldschmidt), nor from a condition in which the cell contained two equivalent nuclei (Lauterborn); but it presupposes the primitive condition to have been a tropho-kinetic binuclearity. These views of Schaudinn have been much elaborated by Hartmann and Prowazek ('07),¹ who have pushed them to their extremest limit. According to these two writers, other protozoan cells are really binucleate in just the same way as the trypanosomes, the only difference being that we usually find the kinetonucleus boxed up—as a karyosome—inside the trophonucleus. The encased nucleus

TEXT-FIG. 24.



Entamoeba tetragena Viereck. *N.*, nucleus, inside which is a "karyosome" with a "centriole" and "a kind of nuclear membrane." This is supposed to represent an encased nucleus. *F.*, ingested body. (After Hartmann and Prowazek, '07.)

assumes many different forms, and it is said in some cases actually to show all the morphological features (centriole, nuclear membrane,² etc.) of a free nucleus (text-fig. 24).

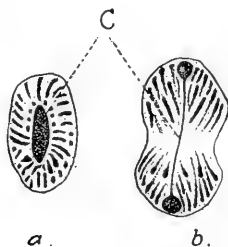
This kinetic nucleus is said to be recognisable in a variety of Protozoa. It is represented by the Nebenkörper of *Paramoeba*, by the Centralkorn of *Heliozoa*, by the nucleolo-centrosome of *Euglena*, by the karyosome in coccidia, etc. It is even suggested that the encased nucleus is visible in a form like *Amoeba limax* (cf. Vahlkampff, '04) but I cannot persuade myself that this is so—with the best will.

¹ They are also held apparently by Keysselitz ('08) and others.

² E. g. in *Entamoeba buccalis* and *E. tetragena*.

This encasement hypothesis is, in face of the facts, to my mind exceedingly far fetched: and moreover, were it true, would not shed any light on the fundamental problem involved. For it is obvious that by assuming the original presence of a separate kinetic nucleus—ancestor of the centrosome—in the cell, we have merely put the problem a little further out of reach. What gives the kinetic nucleus itself the ability to divide? Its centriole? Then is the centriole another kinetic nucleus within the kinetonucleus? And has its own kinetonucleus again inside that, and so on, in an unending box-within-box system? One is forcibly reminded of the “scatulation theory” of the preformationists.

TEXT-FIG. 25.



Sections through the nucleus of *Euglena*: *a*, resting; *b*, in division; *C*, the so-called “nucleolocentrosome.” (After Kenten, '95.)

It is curious to note how a structure like the nucleolocentrosome of *Euglena* can be regarded on the one hand (Goldschmidt, Popoff) as a chromidial apparatus, and on the other (Hartmann, Prowazek) as an actual independent, encased nucleus (text-fig. 25).

There can be little doubt that the karyosome is really a structure of physiological significance in many cases, and, as such, a structure which cannot be homologized throughout the Protozoa. This has been very clearly brought out by Siedlecki ('05) in his admirable study of the coccidian *Caryotropha*. The karyosome, he maintains, is “an amplification of the whole nuclear apparatus.” For him, “we have in a

protozoan cell—no matter whether we see in it a primary nucleus and chromidial mass, or a vegetative karyosome in the nucleus, or even a separate vegetative and generative nucleus—in each case, but a single and simple nuclear apparatus before us.” The physiological nature of the karyosome is also well seen in the case of *Actinosphærium* (cf. Hertwig, '98a). In well-fed animals the karyosome consists almost entirely of plastin, but in ill-fed individuals it comes to be largely composed of chromatin; and so on. Its different behaviour in different organisms is also to be noted. For example, in *Eimeira schubergi* the macrogametocyte casts out the karyosome before fertilisation, whereas in *E. lacazei* it is retained.

That the trypanosome blepharoplast is homologous with the centrosome I have elsewhere ('08b) endeavoured to show. But I cannot in the least agree with the homologization of the blepharoplast with the karyosome. The centrosome, I believe, is an organ of nuclear origin, but originally not a nucleus. The facts regarding the Protozoa and Metazoa¹ all appear to me to point in this direction.

With Boveri ('00) “I fully agree with R. Hertwig in that I do not hold a binucleate condition as the necessary starting point for the phylogenetic origin of the centrosome.”

Phylogenetically, the centrosome probably arose, not from an originally present kintoneucleus, but as a differentiation of part of an original single nucleus—in a manner indicated by Hertwig ('95), Boveri, Calkins, etc. Hertwig himself believed the centrosome to be a specialisation of the central spindle, so that the spindle of Protozoa (e.g. *Paramecium*) is equivalent to centrosome + spindle of the Metazoa. In many groups of Protozoa it is possible to trace a fairly perfect series of nuclear types, from simple amitotic nuclei up to nuclei

¹ Cases of centrosomes appearing in the cytoplasm independently of the nucleus are of course known. But here there is no proof that they did not originally come from the nucleus. (E.g. c f. Yatsu, '05, who admits that the centrosomes do not appear until the nuclear membrane has disappeared.)

dividing by a complex mitosis (e. g. in Flagellata, as I have elsewhere shown, '08).

As Schaudinn ('05) and Prowazek and Hartmann¹ ('07) have pointed out, there can be no doubt that Goldschmidt ('04a) is in error when he describes the blepharoplast and nucleus of *Trypanosoma* respectively as somatic and gametic nuclei. This binuclear condition must, for Goldschmidt, be a secondary one, independent of the real binuclearity (somato-gametic). And conversely, the binuclearity of Infusoria must appear to Hartmann and Prowazek in the same light—as a mere coincidence, having nothing to do with the real trophokinetic binuclearity of the cell. I believe that neither trypanosome nor infusorian represents a primitive condition—both being results of cell differentiation, but along different lines.

Schaudinn's conceptions ('05) did not stop at a trophokinetic binuclearity. He tried to show that there co-exists in the trypanosome cell a sexual binuclearity. There is thus "a double nuclear dimorphism" in these organisms. "The blepharoplast is chiefly male, the large nucleus chiefly female. The dimorphism of both nuclei is hence a sexual dimorphism. The indifferent *Trypanosoma* is hermaphrodite." In *Trypanosoma* the maleness and femaleness find expression in the katabolic nature of the kintonucleus and the anabolic nature of the trophonucleus (cf. the Geddes-Thomson theory of sex). We thus arrive at a conception of the cell as an entity which is partly male and partly female—a conception at which embryologists (Minot, van Beneden, Balfour, etc.) long ago arrived. Schaudinn pointed out that the micronucleus of *Didinium* (Prandtl, '06) must also be regarded as hermaphrodite; and the same is true for Para-

¹ It may be remarked, however, that the occurrence of forms without a trophonucleus in five-day cultures of *Leishmania* no more indicates the function of the blepharoplast than the occurrence of enucleate *Amœbæ* (Prandtl, '07) or gregarines (Kuschakewitsch, '07) proves that the cell does not require a nucleus. In both cases we are probably dealing with degeneration phenomena.

mecium (Calkins and Cull, '07) and probably for other Infusoria. This kind of hermaphroditism must be a very deep-rooted phenomenon if we agree with Schaudinn and his followers (e. g. Prowazek) that sexuality is a fundamental attribute of living matter—a belief which I by no means share.

A view similar to that of Schaudinn regarding the trypanosome cell has been put forward by Salvin-Moore and Breinl ('07), who suggest that the nucleus and blepharoplast are differentiated gamete nuclei in one and the same individual. With Minchin ('08) I believe that this view is "far-fetched and misleading in the highest degree."

In connection with this matter mention may be made of a very remarkable binucleate protozoan, *Amœba diploidea*, recently described by Hartmann and Nägler ('08). The animal contains two nuclei, lying side by side, which a study of the life-cycle has shown to be the two gamete nuclei, which have not fused, from a previous conjugation. Fusion to form a zygote nucleus only occurs before the next conjugation. We have here an organism in which the "paternal" and "maternal" chromatin remain separate all through the vegetative existence. Truly this is a most extraordinary state of affairs. It appears that *A. diploidea* is formed from two incompletely fused organisms, just as *A. binucleata* is formed from two incompletely divided ones.

Finally, I will summarise the conclusions to which the foregoing considerations have led me. They are that the facts relating to chromidia are not yet sufficiently strong to bear the weight of the binuclearity hypothesis which rests upon them: that, therefore, this binuclearity hypothesis, however suggestive it may be as a working hypothesis, is far from being a "law," as some would have it called: and that the tropho-kinetic binuclearity hypothesis is equally unworthy to rank as a cytological truth. The real significance of chromidial structures has been greatly distorted by viewing them from a theoretical standpoint.

The most important inference, however, is that we require

many, many more facts and unbiassed observations before we can hope to unravel the tangled skein of cytological problems of which the foregoing form but a small part. But in the study of the complex simplicity of the Protista we have already found a beginning.

CAMBRIDGE,
August, 1908.

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The Eyes of *Chrysochloris hottentota* and *C. asiatica*.

By
Georgina Sweet, D.Sc.,
Melbourne University.

With Plate 6 and 1 Text-figure.

INTRODUCTION.

After the completion of my work on the eye of *Notoryctes typhlops*, the opportunity was afforded me, through the kindness of Professor R. Broom, of Victoria College, Cape Colony, of examining the eyes of *Chrysochloris hottentota* and *C. asiatica*, which are herein described. I wish, therefore, to thank Professor Broom for my whole supply of material consisting of—

One adult, labelled *Chrysochloris* (*Amblysomus*)
hottentota, Smith.

Two heads of same.

One, three-quarter grown, labelled *C. asiatica*, L. =
C. capensis, Shaw = *C. aurea*, Pullar.

Two young of same: (A) newly born, (B) somewhat older.

The adults of *C. hottentota* and *C. asiatica* were retained as specimens, those two forms being not otherwise represented here.

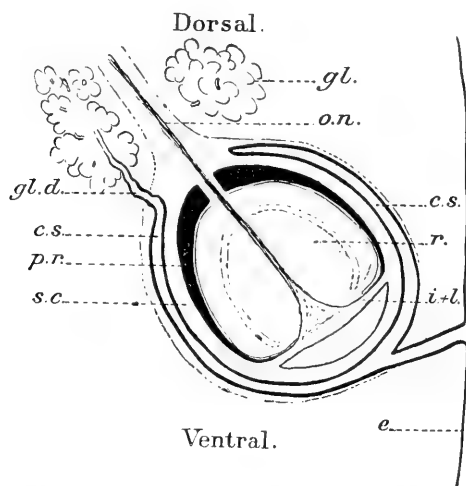
I have also, again to thank Professor Baldwin Spencer for the use of the Biological Laboratory in the University of Melbourne, where this work has been done.

I. *C. HOTTENTOTA*. Adult.

The eyes of this species are situated in the dermis, between the roots of the hairs which surround the eyeball, while

beneath it are the subcutaneous muscles (see Pl. 6, fig. 1). Its general relationship may be seen on reference to the text-figure on this page. It lies at a depth of .138 mm. below the surface, i. e. measured from the front of the sclerochoroid.

Its muscles have quite disappeared. Between the subcutaneous muscles at a short distance behind and on the inner side of the eyeball, is a large serous gland (Pl. 6, fig. 1, *gl.*), the lachrymal.



TEXT-FIGURE 1.—Diagram of a section through the eye of *Chrysocloris*. *c.s.* Conjunctival sac. *e.* Epidermis. *gl.* Gland. *gl.d.* Gland duct. *i.+l.* Iris and lens. *o.n.* Optic nerve-fibres. *p.r.* Pigment layer of retina. *r.* Retina. *s.c.* Sclerochoroid.

The duct of this gland opens into the extreme ventral part of the conjunctival sac. This sac which is quite slit-like, passes almost completely round the whole eye, being absent at the back of the eye only (Pl. 6, figs. 1 and 2, *c.s.*). It communicates with the exterior by a narrow tube almost straight and running outwards, making an angle of 90° with the median longitudinal line of the eyeball, i. e. upwards and backwards. This tube, of course, represents the almost fused eyelids.

The conjunctival sac is lined by a stratified epithelium of two or three layers, similar to that covering the surface of the body (Pl. 6, figs. 1 and 2, *c.*).

The eyeball consists in general of the usual parts found in such degenerate eyes, representing the normal structures of an adult functional eye.

In length, antero-posterior diameter, the eyeball is .51 mm., its transverse diameter being .42 mm.

The fibrous sclerochoroid (Pl. 6, figs. 1 and 2, *s.c.*) is well developed, the front part of the choroid being somewhat less compact than the hinder. The retinal pigment layer (Pl. 6, figs. 1 and 2, *p.r.*) is very well defined; its epithelium of large cubical cells with slightly-staining nuclei almost completely surrounds the eyeball lying just within the sclerochoroid. Its pigmented area is considerably restricted being only present in the posterior one third of the eye, where, however, it has a considerable thickness. It extends somewhat further forwards on the ventral wall of the eyeball (Pl. 6, figs. 1 and 2). There is, however, a gap in the retinal pigment layer posteriorly, nearly in the median longitudinal line, this indicating the region of exit of the optic nerve (Pl. 6, fig. 2, *g.*). The bending in of the pigment epithelium to become continuous with the nervous layers of the retina is very well defined, taking place at about one fifth of the length of the eye from the anterior or outer end. In front of this reflexion, a well-marked structure passes right across the eyeball, leaving an anterior chamber quite separated from the posterior chamber which latter is quite filled with the retina. This degenerate iris (Pl. 6, figs. 1 and 2, *i + l*) arises from the inner layers of the sclerochoroid. It is thin around its origin, but swells out in the centre where it contains an irregular mass of cells in a fibrous-looking matrix; this is evidently the degenerate lens. The rest of the iris consists of a more crowded layer of cells anteriorly, and an areolar layer in the middle.

As the retinal pigment layer passes towards the median longitudinal line of the eye and almost at right angles to it, it becomes associated with a blood-vessel (Pl. 6, fig. 2, *b.v.*)

lined with flattened cells, and branching slightly in the retina. This is also in close relation with the internal fibrous layer of the retina, and with a more or less defined group of ganglion (?) cells. The remaining layers of the retina are comparatively clearly defined, the inner nuclear layer and inner molecular layers are hard to separate, but otherwise the nerve fibre, outer molecular, outer nuclear, and rod and cone layers are all well seen (Pl. 6, fig. 2). The internal and external limiting membranes are often very distinct. The layer of rods and cones separates easily from the pigment layer, owing to the great shrinkage of the retina which takes place after death or during preservation or preparation. Indeed, it appears to me that there may be, in life, a small posterior chamber occupied by some more fluid material than the ordinary vitreous humour contained therein.

In the outer layers of the retina, just opposite the gap in the pigment layer, is a similar space for the exit of the fibres to form the optic nerve.

Dorsally to the median line, the whole retina except the pigment layer is attached to the iris, otherwise lying freely in the optic ball, but passing ventrally the retinal mass is seen to lose this connection and become associated with the back of the eyeball. Near the median line of this area the sclerochoroid and the fibrous layer behind the conjunctival sac begins to be drawn (Pl. 6, figs. 1 and 2), so that the eyeball and surrounding fibrous layer has here a more pear-shaped structure.

At about the same horizontal plane the anterior chamber of the eye is lost, the iris having merged into the wall of the eyeball. Somewhat more ventrally to this again the gap in the pigment and outer retinal layers at the inner end of the longitudinal median line of the eye becomes much bigger and more definite; through it there passes backwards a bundle of optic nerve-fibres. This runs out and down the middle of the fibrous stalk of the now pear-shaped eyeball, this fibrous layer, as it tails out, forming the sheath of the optic nerve.

II. C. ASIATICA. (a) Younger Embryo.

Here the eye lies in the subcutaneous connective tissue, immediately below the dermis and its hair-roots. It is 24 mm. below the surface at its nearest point to the latter.

The usual eye-muscles and associated nerves appear to be quite absent. A small, ill-developed gland is present inside the dermis, close behind the eye, but I was not able to trace any connection between it and the conjunctival sac. The cavity of the latter is represented by a very much reduced hemispherical space, in front of the eyeball. Its external tube does not run direct to the surface, or at right angles to the anterior face of the eyeball, but coils slightly and runs in an oblique direction to the surface, so that light could not penetrate to the bottom of it, unless in a very diffused form.

In size the eyeball is, in its antero-posterior diameter, .48 mm., in transverse diameter .30 mm.

The sclerotic and cornea are again quite similar and not to be distinguished from a choroid—all these three parts are represented by a thin fibrous capsule, which shows no trace of cartilage or pigment at any point. The retinal pigment layer lying immediately within the sclerochoroid is very thick-walled posteriorly, and gradually becomes thinner till, at the anterior part of the eyeball, it is absent for a small space, which may be regarded as the potential pupil. No cellular structure can be seen in the main mass of the pigment.

At the anterior end the reflexion of the pigment layer to become continuous with the rest of the retina is very clear. The space thus left in the middle line anteriorly is occupied by (1) a layer of columnar cells immediately within that part of the sclerochoroid representing the cornea; (2) within this in the middle line is a group of rounded cells bounded by a fine membrane; (3) on either side of this group is a double layer of extremely flattened cells leading out towards the pigment epithelium and sclerochoroid; and (4) immediately

behind (3) are a few irregularly arranged cells. Of these (1) appears to represent the outer layer of the iris; (2) this group of cells is evidently a degenerate lens; (3) is the remnant of the inner layer of the iris and the group of cells; (4) appears to represent some ganglion cells. Within the pigment ball lies the mass of retinal cells, showing very little differentiation into layers, and completely filling the cavity of the eyeball, there being visible no vitreous humour or lens (other than perhaps the group of cells mentioned above).

The outermost layer of cells is very densely packed, the cells having deeply-staining nuclei being apparently the outer nuclear layer. Between this and the pigment layer, and generally embedded in the latter, is a clear, almost homogeneous layer, probably representing the degenerate layer of rods and cones.

Within the outer nuclear layer the cells are much less closely packed, especially in the centre of the mass, but no clear division into the usual layers is present.

Along the median antero-posterior line is an irregularly double line of much flattened cells, associated with a thin fibrous layer, possibly representing internal limiting, and hyaloid membranes with nerve-fibre layer—the vitreous cavity being lost. No definite nerve-fibres are visible.

(b) Older Individual.

In this specimen the eye does not vary to any remarkable extent from that of the younger forms. Its depth below the surface is greater than in (a), being .54 mm., though it appears relatively less deeply situated, owing to the greater depth to which the hair-roots extend.

The conjunctival sac extends almost completely round the eyeball, being only absent at one spot on the postero-ventral aspect—no doubt representing the place of exit of the optic nerve. Its walls are much more fibrous than in (a). The sac does not appear to open to the surface in one eye of this

embryo, its duct towards the exterior extending only a very short distance in front of the sac. That belonging to the other eye is, however, much more well-defined, and has a duct clearly leading on to the surface of the head. I have been unable to find any gland in this stage.

The eyeball is somewhat more elongated than that in the younger stage, being .62 mm. in antero-posterior diameter, and .44 mm. in transverse diameter.

The fibrous sclerochoroid is similar to that previously described, except that it is more strongly developed, and the choroid portion is less densely fibrous than the sclerotic part. The retinal pigment epithelium is well defined, though its pigmented area, as in *C. hottentota*, is in one eye (left) of this young individual much less extensive than in (*a*), being confined entirely to the posterior half, except in the median longitudinal line, where it extends round the front also. In the other eye (right) of this specimen the pigment is much more abundant, being only absent from the layer of pigment cells over a small circular area at the anterior end of the eye, much as in (*a*). In both eyes this pigment cell layer is absent in the median longitudinal line posteriorly, as in *C. hottentota*.

At the anterior end of the eye in this stage also the continuity of the pigment layer with the other layers of the retina is clearly seen, the reflexion taking place at about one sixth of its length from the front of the eye.

Passing over the anterior face of this layer is a membrane representing probably both internal limiting membrane and hyaloid membrane. This is continued back along the median longitudinal line to the level of the posterior gap in the pigment cell layer. There are associated with it remnants of optic nerve-fibres, but these are extremely indefinite, and are not continued outside the eyeball, there being no semblance of optic nerve.

At the anterior end of the eye, filling the triangular space which is left in the median line in front of the reflexion of the pigment layer, and which one may regard again as the

pupil, is a mass of cells and fibrous tissue irregularly arranged. This is the much more degenerate iris and lens.

The retinal layers have completely filled the greater part of the eyeball, so that in section the internal limiting and hyaloid membranes from either side are seen to be in contact along the median longitudinal line of the eyeball, there being no vitreous humour or posterior chamber. In more favourable parts, the outer nuclear, outer molecular, and inner nuclear layers are clearly to be seen, but the remaining layers are very ill-defined. No special ganglion cell layer is observable.

Outside the outer nuclear layer is an irregular, staining very faintly indeed, but often visible where the retina is torn away from its pigment layer. Distorted rod-like structures may be detected here and there in this, which is evidently all that is left of the layer of rods and cones.

(c) Adult.

The eye of the adult of *C. asiatica* lies as usual in *Chrysochloris* in the dermis among the roots of the hairs which surround the eyeball, while beneath it are the subcutaneous muscles.

The distance of the anterior face of its corneal region from the surface is .51 mm. The length of the eyeball is .54 mm., and its transverse diameter .38 mm.

Its gland mass is much more deeply situated than in previous forms, the gland duct to the conjunctival sac being wide and very definite. No tube was to be found with certainty opening to the surface, but in view of the well-developed character of the gland-duct and conjunctival sac, and of the fact that no other exit was apparent for the excretion, it is probable that it was torn or distorted in some way during preparation, so hindering its detection.

In almost every detail otherwise, the eye of the adult of *C. asiatica* is closely similar to that of *C. hottentota*.

SUMMARY OF STRUCTURE.

1. The eye has sunk only into the dermis being surrounded by the hair-roots.

2. The conjunctival sac is well developed and also generally the lachrymal gland, the duct of which opens into the sac. From the sac, in most cases a cylindrical tube leads to the exterior. This tube, however, from its direction and sometimes coiling, can be of no use as a path for light rays giving rise to vision.

3. The eye muscles are quite absent.

4. Sclerotic cornea and choroid are represented by the fibrous sclerochoroid.

5. Lens and iris are very degenerate though recognisable. The vitreous humour is absent.

6. The pigment layer of the retina is thick posteriorly, and absent anteriorly.

7. The retinal layers are, in most cases, clearly distinguishable, very little degeneration being apparent in the outer ones. The layer of rods and cones is more or less distinct.

8. The optic nerve is present in some, viz. the two adults and older immature form, though the ganglion cell layer is the most degenerate part of the retina.

COMPARISONS AND CONCLUSION.

As regards the position of the eye, that of *Chrysochloris*, while not generally visible from the exterior, even after shaving off the hair, as it is in *Scalops* (Slonaker, p. 335) and *Talpa* (Kohl, '93, '95, p. 13), is still comparatively superficial in contrast to that of *Rhineura* (Eigenmann, '02, p. 535) and *Notoryctes* (Sweet, p. 549).

The eye muscles are here absent, in contrast to most other burrowing forms, e.g. *Scalops*, *Talpa* (loc. cit.), *Typhlops* (loc. cit., and Kohl, '92, p. 124). The space between the conjunctival layers and between the eyelids is repre-

sented by the conjunctival sac, and generally a cylindrical tube to the exterior, as in *Scalops*, *Talpa*, etc.

The gland mass is generally well developed, as in *Typhlops* (loc. cit., p. 119, etc.), and *Notoryctes* (loc. cit., p. 551, etc.), its ducts opening into the reduced conjunctival space. Instead of the secretion being passed into the mouth as in *Typhlops* (loc. cit., pp. 119—121), or the nose, as in *Notoryctes* (loc. cit., pp. 552—554), it here usually runs out to the exterior directly through the "eye-cleft" as in *Scalops* and *Talpa*.

The sclerochoroid is similar to that in other degenerate eyes. The iris and lens are much more degenerate than in *Scalops* (loc. cit., Pl. 18, fig. 5, and Pl. 19, fig. 9), *Talpa* (loc. cit., p. 26, and Taf. III, figs. 27, 28, etc.), or *Typhlops* (loc. cit., Taf. VIII, fig. 84), but greatly less so than in *Notoryctes* (loc. cit., p. 559), where they are not recognisable as such at all.

The pigment layer is apparently not reinforced by degeneration to any great extent, and, as in most degenerate vertebrate eyes (except *Notoryctes*, *Troglichthys* [Eigenmann, '99⁽¹⁾, p. 581], and *Typhlomolge* [Eigenmann, '99⁽²⁾, p. 53], etc.), is thickest behind, and more or less absent in front.

The retina is much more highly developed than one might expect from the condition of the lens, iris, etc.

The variability so often shown by degenerating structures is very evident here, not only between individuals, but also between opposite sides of the same individual, e.g. in the degree of pigmentation present in the retinal pigment layer, in the length and clearness of the optic nerve-fibres, and in the completeness of the severance of the connection with the exterior.

In comparing the eye of *Chrysochloris* as a whole with that of *Talpa*, *Scalops*, and *Notoryctes*, it may be stated in general terms that it is distinctly more degenerate than the eye of *Talpa* or *Scalops*, but very much less so than that of *Notoryctes*.

It is interesting to note that placing the individuals herein

investigated in order of degeneration, we find that the adults of *C. hottentota* and *C. asiatica* are the least degenerate, the intermediate stage of *C. asiatica* being the most degenerate in nearly every respect.

The eye of *Chrysochloris* is, without doubt, of no use for vision, even were the eye-cleft at the proper angle to admit light-rays in the proper direction, i. e. along the optic axis, and it is improbable that even degrees of light can be detected by it. At this stage of degeneration the gland secretion can have only its usual function of keeping the conjunctival cavity free from foreign matter.

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I regret that I have been unable to consult any literature on *Chrysochloris* other than one or two purely systematic papers, the valuable work done by other workers on this animal not being obtainable in Australia.

EXPLANATION OF PLATE 6,

Illustrating Miss Georgina Sweet's paper on "The Eyes of *Chrysochloris hottentota* and *C. asiatica*."

REFERENCE LETTERS.

b.v. Blood vessel. *c.* Conjunctiva. *c.s.* Conjunctival sac. *d.* Dermis. *e.* Epidermis. *g.* Gap for optic nerve. *g.h.* Group of hairs. *gl.* Gland. *gl.d.* Gland-duct. *i + l.* Iris + lens. *i.n.l.* Inner nuclear layer. *m.* Muscles. *o.m.l.* Outer molecular layer. *o.n.* Optic nerve fibres. *o.n.l.* Outer nuclear layer. *p.r.* Pigment layer of retina. *r.* Retina. *r.c.* Layer of rods and cones. *s.c.* Sclerochoroid. *s.o.n.* Sheath for optic nerve.

All figures were drawn with the aid of the camera lucida.

FIG. 1.—General view of dermis of *C. hottentota* with eyeball, showing relations of latter.

FIG. 2.—More highly magnified view of eyeball of *C. hottentota*, showing more detailed structure. Compiled from several consecutive thin sections.

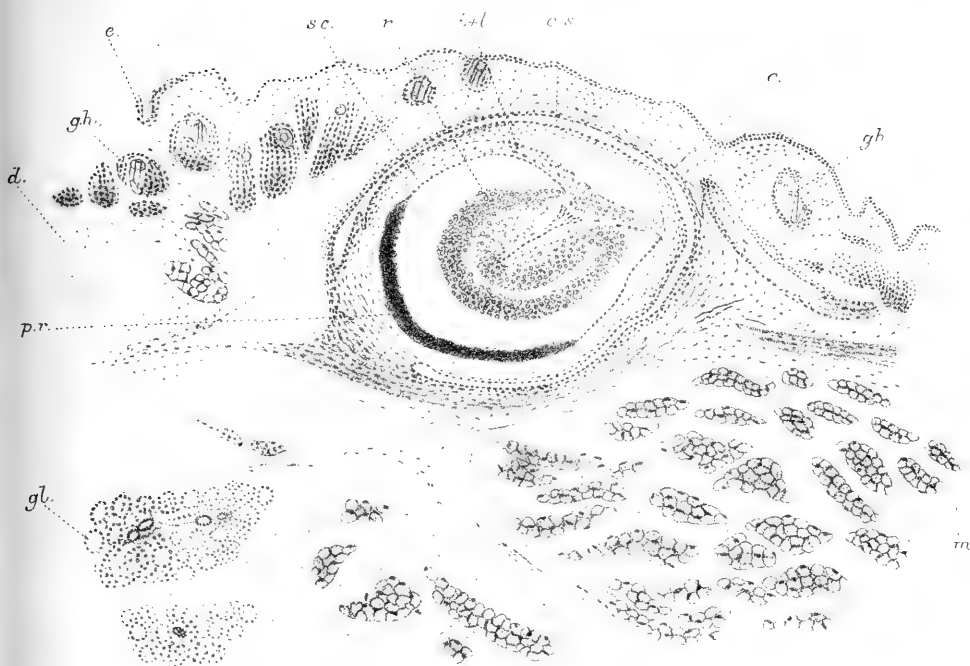


Fig. 1.

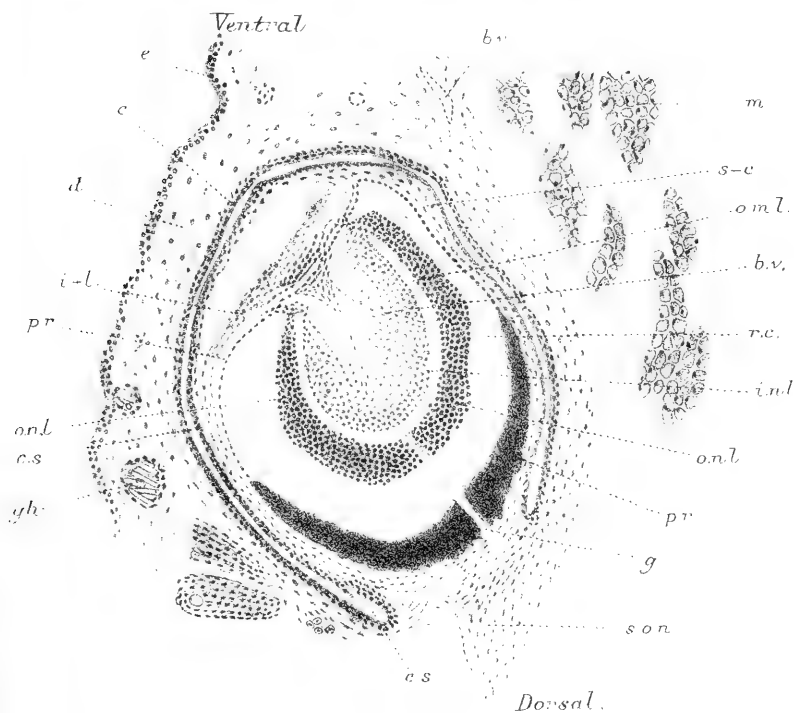


Fig. 2

**On the Occurrence of Nuclear Dimorphism in a
Halteridium parasitic in the Chaffinch, and
the probable connection of this parasite with
a Trypanosome.**

By

H. M. Woodcock, D.Sc.Lond.,

Assistant to the University Professor of Protozoology.

IN the course of an investigation on the Hæmatozoa of birds, undertaken as Mackinnon Student, I have recently observed certain forms which are of great importance in reference to Schaudinn's view of the ontogenetic relationship between a Trypanosome and a Halteridium of the "Little Owl."

The material was furnished by a chaffinch, known to be infected with a Trypanosome, which was found to be heavily infected with Halteridium also, towards the end of last June. The preparations of which I shall take account in this note were all made between 1 and 3 a.m.; they comprise smears from the peripheral blood, the heart-blood, and from most of the organs. Unfortunately the lungs were forgotten—an omission which I have deeply regretted. The smears were all well fixed with osmic vapour and stained by some modification of the Romanowsky method. Some of the preparations of the peripheral blood were left for a few minutes before smearing, with or without the addition of a drop of salt-citrate solution. As a result, in these smears free fully-formed microgametes and free rounded-off female elements can be more or less readily found.

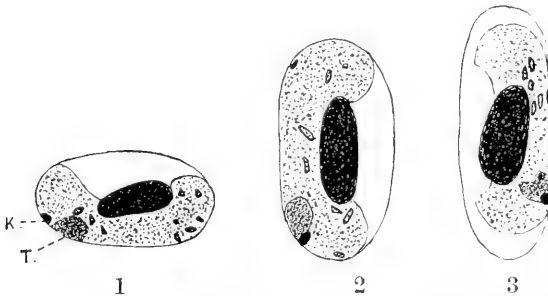
The Halteridia being so numerous in this bird at that time, I confess I expected, from Schaudinn's account, to have little or no difficulty in finding various stages in the transition of the parasites from an active, trypaniform phase, to a resting, intra-cellular, Halteridium-phase and vice-versâ—always supposing, that is, such a connection exists in this case. A general examination of the more likely slides, including smears from the bone-marrow, etc., showed no indications of such behaviour. Trypanosomes of any kind were very scarce as compared with the great number of Halteridia present, and the few individuals noticed were manifestly larger than the largest Halteridia. I was unable to do more than make a somewhat cursory examination at the time, as I was very much occupied with the research in another direction; and owing to the pressure of other work subsequently, it was not until autumn that I was in a position to begin the laborious and time-consuming process of systematically searching these slides. I am pleased to say this study has now yielded me some most interesting results, and further search would yield, I believe, more yet. As, however, I shall probably have to leave the work at this point for some time, I think it worth while to publish this note. I propose to state shortly those observations made up to the present which bear upon the above question, and to consider briefly the meaning which, it seems to me, is to be attached to them.

Fully-grown Halteridia of three types occur, male and female forms with the usual well-known distinguishing features, and a third type corresponding to the "indifferent," or non-sexual form of Schaudinn; the last-named type is distinguished from a female form by its much lighter staining cytoplasm, and from a male form by its compact, denser nucleus. This indifferent type is by far the least common of the three in these slides. Halteridia of all sizes, however, are to be found in the red blood-corpuscles, from very minute, oval or pear-shaped forms, 2μ or less in diameter, up to the large adult individuals. Many different phases can

often be seen in a few fields of the oil-immersion lens, particularly in liver-smears. A very large number of *Halteridia* have passed under my eye, but I have never seen the least sign of endogenous multiplication (schizogony) in any of the adult individuals in the red blood-corpuscles, i. e. never anything approaching the nuclear fragmentation and segmentation of the cytoplasm at the two ends, which was described by Labbé.¹ The minute forms do not arise, I am convinced, by the division of the large ones.

Many of the *Halteridia* exhibit in regard to their nuclear

TEXT-FIGS. 1-3.



Female individuals of *Halteridium*. 1. From peripheral blood, left a couple of minutes before smearing. 2. From liver-blood; and 3, from peripheral blood (of living bird), both smeared at once. *K*. Kinetonucleus, or kinetonuclear element. *T*. Trophonucleus, or trophonuclear element. Fig. 1 $\times 2000$; Figs. 2 and 3, $\times 2500$.

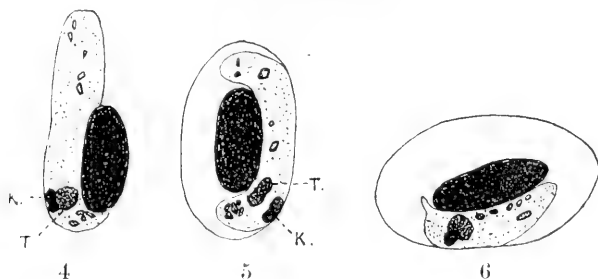
structure a condition which undoubtedly represents nuclear dimorphism. By the term "nuclear dimorphism" is understood a characteristic separation of the nuclear material into two constituents, a larger body staining red with Romanowsky modifications, and a smaller one, which is much denser and stains much darker. These two nuclei, which I have distinguished² as tropho- and kinto-nucleus respectively, are of regular occurrence in Trypanosomes and other parasitic

¹ 'Arch. Zool. Exp.,' ser. 3, vol. 2, p. 55, 1894.

² "The Hæmoflagellates," 'Quart. Journ. Micr. Sci.,' vol. 50, p. 151, 1906.

Flagellates. I first observed this condition in some of the large female individuals, in which it appears as seen in text-figs. 1-3. Lying close to the ordinary nucleus, generally in contact with it, is a much smaller body, which stains much more deeply than the large one, at times appearing almost black (*K.*). This little body is generally ovoid or round, but in some cases tends to have a rod-like shape. There is no possibility of confusing this nuclear body with a large pigment-grain, or a collection of grains. It lies usually near to the outer surface of the body. In parasites of the indifferent type this nuclear element, which I homologise with a kinetonucleus, is larger

TEXT-FIGS. 4-6.



Indifferent forms of *Halteridium*. 4 and 5. From heart blood, smeared straightway. 6. From peripheral blood, after addition of a drop of salt-citrate solution, and interval of a couple of minutes before smearing. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. $\times 2500$.

and may approximate to the size of the other nucleus (trophonucleus). It may be round (text-fig. 6) or, frequently, it is more or less dumb-bell-shaped (text-fig. 5), as if it were composed of two halves. It is important to note that this nuclear dimorphism can be readily recognised in many of the minute forms, the kinetonucleus being a small, deeply-staining grain lying close to the ordinary nucleus, generally at one side (text-fig. 12). This feature gives several of these small individuals a striking resemblance to the resting-phases described of various *Herpetomonadine* parasites.

In none of the male forms scrutinised so far have I been able to make out a distinct kinetonuclear body. This may be because it is not differentiated, as a compact organella, from the rest of the diffuse nucleus of this type. (I am inclined to think, however, that it is present in the free, fully-formed male gametes, although it is difficult to feel quite sure; I shall refer again to the structure of these delicate elements.) Moreover, in many of the female individuals, the kinetonuclear element is by no means so prominent or separate as in the examples figured, which have been chosen to show this feature as clearly as I have observed it. Others, again, do not show it at all. It is quite probable that at times the two

TEXT-FIG. 7.



Free form, from heart-blood; smear made at once. $\times 2500$.
(Probably the upper nuclear body is the kinetonucleus.)

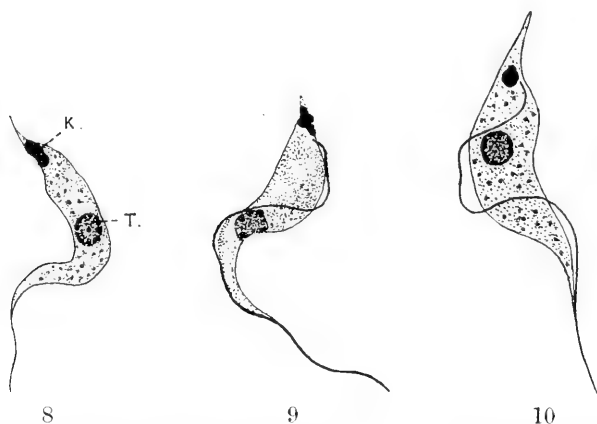
constituents are incorporated in one nucleus, as was said, indeed, by Schaudinn to occur at certain periods.

It is highly significant, I think, that up to the present, when an intra-cellular parasite has been known to exhibit nuclear dimorphism, although lacking in that stage any flagellum or obvious sign of Flagellate affinity, it has been subsequently found to be really a phase in the life-cycle of some Flagellate form; in other words, the knowledge of nuclear dimorphism in a parasite has hitherto heralded, as it were, the discovery of its intimate connection with a Flagellate. The classic instance is the Leishman-Donovan body, whose flagellar phase was first made known by Rogers. Again, Schaudinn himself maintained that certain Piroplasmata showed this character, and others (e.g. Lühe) have since corroborated him. Only recently Miyajima¹ has carried the

¹ 'Philippine Journ. Sci.,' ser. B, vol 2, p. 83, 1907.

matter a step further, and obtained the development of unmistakable Trypanosome phases in cultures from a Piroplasma of cattle in Japan. Hence, the occurrence of this feature in Halteridium even regarded by itself is, to my mind, most suggestive; and it is with very great pleasure that I bring forward what is, I believe, the first definite piece of evidence tending to confirm one, at all events, of Schaudinn's celebrated conclusions.

TEXT-FIGS. 8-10.



Trypanosomes from a bone-marrow smear. In 8 the flagellum is very faintly stained, and its course along the side of the body cannot be followed. K. Kinetonucleus. T. Trophonucleus. $\times 2500$.

Stimulated by this discovery, I have striven to find some phases showing the actual passage from a Halteridium-form to a Trypanosome-form, but in the case of the parasites of the chaffinch such phases appear to be very few and far between. This is, unfortunately, only what might be expected from the great scarcity, comparatively, of the Trypanosomes themselves. I have obtained certain indications, which, so far as they go, point to such a transformation, or fit in with it; and I have observed nothing which in any way invalidates this view.

Before leaving the Halteridium side of the question, there is one phase which I have found, to which I attach con-

siderable importance (text-fig. 7). This is in a smear, well fixed and well stained, which was made from heart-blood and smeared straightway. The parasite is not altered or deformed in any way mechanically; I am confident that it represents a normal phase. The individual in question is free in the blood. It is of the indifferent type, and the two nuclei are in close contact, both dense and deeply staining. The pigment grains are all near one end of the cytoplasm, in

TEXT-FIG. 11.

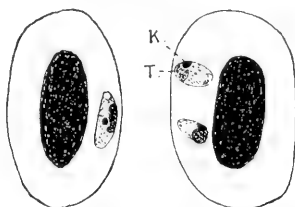
Large Trypanosome, from peripheral blood. $\times 2500$.

a position in which they might easily be got rid of. The most interesting point is the presence of an unmistakable thread or line, which is stained bright red. This starts from a short, transverse, deep-staining band, adjoining the two nuclei, and runs down part of the length of the body near to one side, terminating in a definite granule. The narrow portion of the cytoplasm between it and the margin of the body has a distinctly reddish tinge, the rest of the protoplasm being of the usual blue colour. The only explanation I can give of this thread is that it represents the "central spindle"

described by Schaudinn, which in later development becomes the flagellar border of the membrane, i. e. the proximal part of the flagellum.¹

Turning to the question of the Trypanosomes, I have now succeeded in finding, in this series of slides, particularly in smears of the bone-marrow, a few very small Trypanosomes, forms which are no larger than the large individuals of *Halteridium*. When these little Trypanosomes (as in text-figs. 8 and 9) are compared with the large forms in the blood at this time (e. g. fig. 11), the difference in size is seen to be very marked. Between these extremes all intermediate

TEXT-FIG. 12.



Small forms of *Halteridium*, from a liver-smear. The right-hand figure shows the appearance when two parasites are in one corpuscle, which is of frequent occurrence. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. $\times 2500$.

stages can be found. I could figure a series of regular gradations from the one to the other. Now, I have never seen the slightest indications of division; even at this time, when the Trypanosomes are less infrequent than at other times, e. g. early spring or late autumn. Hence, I consider that the larger forms have grown from smaller ones, and not that the small ones have arisen, by successive multiplication, from the large individuals. With regard to the origin of the very small Trypanosomes themselves, I am certainly disposed to think that they arise from adult *Halteridia*. This seems to me to be the most reasonable conclusion to arrive at, having regard to the data I have ascertained so far.

¹ I have now found this phase in two or three instances.

It is true that the number of Trypanosomes is very small in proportion to that of the Halteridia, but the disproportion is largely reduced if the sexual forms of the intracellular parasites are left out of account; and, as I have mentioned, the great majority of the Halteridia are of the male or female type. It is probable that the Trypanosomes are reinforced in number from the indifferent Halteridia as a rule; and from the female forms only in exceptional circumstances, e. g. perhaps late in the season, if unable to become fertilised. I do not think it likely that the male individuals give rise to ordinary Trypanosomes at all.

In some of my preparations very good examples of micro-

TEXT-FIG. 13.



Male gametes, from peripheral blood, left a couple of minutes before smearing. *b* is the least intensely stained, *c* the most so. *g.* Centrosomic granule. $\times 2500$.

gametes are to be found. These had, in life, freed themselves from the residual body of the gametocyte, were actively motile, and for all I know to the contrary were fully-developed male elements. They have been examined under the best obtainable conditions. This is essential, since the width of these fine organisms is only from $\cdot 5$ to $\cdot 7 \mu$. Unfortunately, too, the gamete takes up the stain so intensely, that it is often difficult to differentiate sharply between the nuclear and cytoplasmic portions. The chief evidence of trypaniform structure which I hoped to obtain was, of course, the presence of an undulating membrane, as shown by Schaudinn in his schematic figure. I examined, particularly, deeply stained specimens, thinking the flagellar border, standing out from

the body, would be more distinguishable in such. I was not able, however, to satisfy myself of the existence of such an organella. I am uncertain whether there is one or not; it may be that I have not been able to discern it owing to its contiguity to the general cytoplasm.

In certain respects the structure of these gametes does undoubtedly agree with that described by Schaudinn. At one end, which is abruptly rounded, there is an unmistakable granule, which stains more deeply than the neighbouring cytoplasm (text-fig. 13, *g.*). This organella is apparent in most of the individuals observed, and most probably corresponds to the anterior centrosome of Schaudinn. The opposite end is

TEXT-FIG. 14.



Dividing parasite, probably giving rise to small Halteridia; from the bone-marrow. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. \times 2500.

finely tapering, and comparable to the cytoplasmic tail. I have not been able to make out any elaborate nuclear details, but I am inclined to think a kinetonuclear body can be distinguished; at all events, one of the chromatic masses, of which there appear to be three or four, is usually larger and more deeply-staining than the others (cf. fig. 13).

In conclusion, I have a few words to add with regard to the origin of the minute intra-cellular Halteridia. Two or three weeks after this series of preparations was made, I saw for the first time Aragao's account¹ of his work on the Halteridium of the pigeon. Aragao has found that the very young forms, which enter the red blood-corpuscles, originate by the multiple division (schizogony) of large parasites which occur in the endothelial cells of the lung-capillaries. This worker thinks that the form or phase

¹ 'Arch. Protistenk.', 12, p. 154, 1908.

—whatever it may be—in which the Halteridial parasite enters the blood from the Invertebrate host, in this case a *Lynchia*, passes first into one of these endothelial cells. In this position it grows and subsequently gives rise to a progeny of little individuals, which, when set free, penetrate the red corpuscles and become the well-known Halteridia.

I have no doubt a similar process occurs in the case of the parasites of the chaffinch. Most unfortunately I omitted to make smears from the lungs. In a smear from the bone-marrow, however, I have come across two instances of a phase which, I believe, corresponds to the segmenting forms described by Aragao. One of these parasites is drawn in text-fig. 14. It is free, having evidently broken loose from the cell, perhaps a leucocyte, in which it was parasitic. It is in process of multiple division, possessing several nuclei. The most interesting point to notice is that some of these nuclei show nuclear dimorphism. Associated with the larger, more obvious nucleus is a small, deeply-staining grain, which probably represents the kinetonuclear element. Two or three of these "double" nuclei closely resemble the double nuclei of the minute Halteridia in the red blood-corpuscles (cf. text-figs. 12 and 14). Unfortunately, the specimen is rather darkly stained, and I cannot be sure of this feature in all the daughter-nuclei.

The connection of *Halteridium* with a parasite of cells other than red corpuscles (endothelial cells, leucocytes, etc.), made known by Aragao, furnishes another link in the complicated chain of events, which, according to all indications, make up the complete life-cycle of this form. It is instructive to note that *Leishmania donovani*, which is now admitted by everyone to be the intra-cellular phase of a Flagellate, is a parasite of such cells; and it is not yet certain whether it invades the red corpuscles. *Halteridium* is probably a stage in the life-history of a Trypanosome, which has advanced a step further and become adapted also to the red corpuscles.

THE LISTER INSTITUTE,

December 10th, 1908.

Some Observations on Acinetaria.

By

C. H. Martin, B.A.,

Demonstrator in Zoology at Glasgow University.

With Plates VII and VIII.

Part I.—The “Tinctin-körper” of Acinetaria and the Conjugation of *Acineta papillifera*.

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I. INTRODUCTORY.

The observations described in this paper were begun on a culture of *Tokophrya elongata*, which was given to me at Munich by Professor Hertwig in the summer semester of 1902. This culture was followed for a period of about six months, when it was unfortunately lost, but as during this time I had never seen any indication of conjugation, I thought

it better to place the results I had obtained on one side until the occurrence of a more favourable opportunity.

During the early summer of 1907, whilst working in Norfolk, I found an Acinetarian which corresponded with Keppen's *Acineta papillifera*, in great abundance on the stems of *Cordylophora lacustris* in Hickling Broad. During the early part of September an epidemic of conjugation set in, and although, owing to the pressure of other duties, I was not able to follow the stages in the living form as completely as I should have wished, I was able to obtain enough fixed material to work through the main nuclear changes in this process. In the second part of this paper the life-history of a new Acinetarian parasite *Tachyblaston ephelotensis* is described, and in a future paper I hope to deal more fully with some general questions as regards the mechanism of the tentacles, as well as the relations between the lageniform and proboscidiiform individual in that remarkable animal *Ophryodendron*.

I should like to take this opportunity of thanking Professor Hertwig, to whom the inception of this work is entirely due, for the great kindness he showed me at Munich, and Professor Graham Kerr for allowing me to work through my material in the laboratory at Glasgow University.

II. THE "TINCTIN-KÖRPER" OF ACINETARIA WITH SOME OBSERVATIONS UPON THE NUCLEAR CHANGES OF *TOKOPHYA ELONGATA*.

Before proceeding to give an account of the conjugation of *Acineta papillifera*, it will be necessary to give some account of those isolated masses of chromatin, the "Tinctin-körper" of Plate, which may occur in all free-living Acinetarians, and which enormously increase the difficulties of work upon the nuclear changes during conjugation.

Plate in 1886 first drew attention to certain rounded bodies occurring in the cytoplasm of *Dendrocometes* which were

not blackened by osmic acid, and which were stained by safranin, and also, though less brilliantly, by carmine.

He called these bodies "Tinctin-körper," and noted that in some individuals they seem to be altogether absent.

When present they are usually more or less rounded bodies measuring up to .006 mm., but occasionally they assume "eine wurst-formige unregelmässig längliche Gestalt und erreichen dann auch eine viel beträchtlichere Grosse."

At first he was inclined to believe that these bodies were equivalent to the micronuclei of the Ciliates, but he concludes by stating that the present state of our knowledge "lässt es rathsamer erscheinen sie vor der Hand nur als eigenartige Produkte des Stoffwechsels anzusehen."

Schneider also observed these structures in *Stylocometes*, but was inclined to believe that the "Tinctin-körper" are the products of the degeneration of the old macronucleus after conjugation.

This view is also upheld by Bütschli (in 1889) "Ich halte diese Ansicht für recht wahrscheinlich um so mehr, als wir ja auch bei den Ciliaten erfuhren, dass die Fragmente des Alten Ma. N. häufig sehr lange erhalten bleiben, und bei der Theilung auf die Nachkommen übergehen können, wie es für die Tinctinkörper der *Dendrocometinen* gilt."

Sand (1901) in his monograph puts forward the view (p. 31) that "lorsqu'un Acinétien suce une proie, le sarcode de celle-ci, dès son entrée dans le cytoplasma du Tentaculifère s'amasse en sphères de mêmes dimensions que les sphères de tinctine; comme le cytoplasma mort est très colorable, et que du reste, les sphères contiennent, outre le cytoplasma le noyau de la proie, elles se colorent fortement par les teintures nucléaires."

Personally I believe that under the name of "Tinctin-körper" chromatic granules which may originate in one of three distinct ways are confused—those derived

- (1) From the ingested nucleus of the prey,
- (2) From the degenerating macronucleus after conjugation,
- (3) Possibly to a very slight extent in some cases from

fragments of the macronucleus thrown out in connection with the formation of a digestive ferment.

Of these three modes of origin the first is by far the most common since conjugating *Acinetaria* are relatively quite rare. But it would still seem necessary to retain the general term "*Tintin-körper*" since practically these bodies are only distinguishable when the details of the past life of the individual under examination are known.

Tokophrya (Bütschli) *elongata* (Clap. et Lachm.) is a more or less cylindrical *Acinetan* provided with a short stalk and with tentacles scattered in rather indefinite groups over the surface of the body.

It is an exceedingly easy form to cultivate, as it feeds readily upon almost all *Ciliates*, and appears to have few enemies.

My cultures were kept in watch-glasses, to the bottoms of which the young *Acinetarians* fixed themselves by their stalks; they were generally fed upon *Stentor*, but when the supply of *Stentor* was short they seemed to thrive equally well on *Paramecia*.

When the *Stentor* came in contact with the tentacles of the *Acinetan*, after some short struggles the cilia of the former ceased to beat and the animal remained paralysed.

As the *Acinetan* was very much smaller than its prey the first attack was rarely fatal, but if, as generally happened in a healthy culture, a single *Stentor* was attacked by five or six *Acinetarians* the whole animal would disappear at the end of a couple of hours.

To the details of this process and the mechanism of the tentacles I hope to return in a future paper in which I wish to describe certain feeding experiments made on *Ephelota gemmipara*.

Tokophrya elongata reproduces itself by the formation of internal hypotrichous ciliated buds, which escape through "the birth opening"—a lateral slit in the pellicule.

In a normal *Tokophrya elongata* the macronucleus is a band-shaped structure passing down the long axis of the

body, but in addition to this there are always scattered in the cytoplasm a number of granules of chromatin, the "Tinctin-körper."

There seem to me to be three lines of evidence showing that the ordinary "Tinctin-körper" cannot be regarded as integral parts of a fragmented macronucleus—

(1) From their behaviour during reproduction and conjugation.

(2) From their behaviour during starvation and feeding.

(3) From their absence in parasitic forms such as *Tachyblaston ephelotensis* to be described later.

(1) There is no trace of a connecting thread between the "Tinctin-körper" such as is present in the macronuclei of *Stentor* and *Holophrya*, and there is no reunion to a single mass previous to division, such as has been described for some *Hypotrichous* Ciliates with a fragmented macronucleus.

In dividing *Tokophrya* the macronucleus becomes twisted upon itself in the region of the future bud, but in all the stages of division isolated "Tinctin-körper" are to be found both in the cytoplasm of the mother and of the bud (Pl. VIII, fig. 2).

In conjugating *Acineta papillifera* at stages at which the macronucleus was still intact individuals were found in which the "Tinctin-körper" seemed to be collected in the lower part of the theca, quite apart from the macronucleus.

(2) Their Behaviour during Starvation and Feeding.—During starvation there is a tendency for the "Tinctin-körper" to disappear, though even in the most complete cases (Pl. VIII, figs. 3 and 4) some remains were present. In Pl. VIII, fig. 4, which represented the last stage of starvation, the individual measured 25μ in length and 18μ in breadth, a normal well-fed *Tokophrya* from the parent culture measuring 143μ by 58μ . The tentacles and most of the cytoplasm had disappeared, but the nucleus seemed quite healthy staining rather more readily than in the well-fed forms.

In preparations made a quarter and half an hour after

feeding slightly-starved *Tokophrya* upon *Stentor* the "Tinctin-körper" showed no marked increase over the control form, and it is interesting to note that up to this stage the nuclei of the *Stentor*, although slightly swollen, had not been ingested. But in individuals which had ingested the nuclei of the prey enormous masses of "Tinctin-körper" were found resulting in such an appearance as is figured in Pl. VIII, fig. 1, in which the whole of the cytoplasm is blocked with "Tinctin-körper."

It is remarkable how little attention has been hitherto paid to the possible presence of ingested chromatin in Protozoa, although it is evident that unless the process of digestion is extremely rapid, these ingested masses may quite easily form a considerable source of error in the description of the nucleus of any holozoic protozoon.

As far as I am aware the only careful description of the appearance and behaviour of ingested chromatin in a Protozoon is to be found in Schaudinn's account of *Trichosphærium sieboldii*. Schaudinn (p. 81) found that in *Trichosphærium* the nuclei eight hours after ingestion were not greatly changed; in later stages the linin is dissolved, and the chromatin sinks to the lower side of the nucleus. "Das Chromatin wird nun auch allmählich gelöst, und nimmt hierbei meist Kugelgestalt an. Es schien mir, als ob hierbei seine Farbbarkeit zunimmt was vielleicht darauf beruht, dass bei der Verdauung ein nicht färbbarer Theil seiner substanz früher gelöst wird, während die färbbarer Theilchen dichter zusammengedrängt werden und daher in ihrer Gesamtheit dunkler gefärbt erscheinen."

As regards the details of this process of digestion in *Acinetaria*, the earlier stages are passed through whilst the nucleus is still lying in the cytoplasm of its prey. But in the early stages of the degeneration of the macronucleus of a conjugating form quite analogous early stages of digestion are to be found in the cytoplasm of the *Acinetarian*.

The later stages of the digestion as figured by Schaudinn

have an absolutely identical appearance to the "Tinctin-körper" of the Acinetaria.

(3) In a parasitic form, *Tachyblaston ephelotensis*, which I describe in the second part of this paper, I could never find any trace of "Tinctin-körper."

It is interesting to note that in this case the nucleus of the host was never ingested by the parasite.

From a consideration of these points it will, I think, become evident that, in the great majority of cases, the scattered chromatin granules found in Acinetaria can only be regarded as the partially digested nucleus of their prey.

III. THE CONJUGATION OF ACINETA PAPILLIFERA.

Without attempting to give a history of our knowledge of conjugation in the Acinetaria (which has already been fully done by Bütschli in his great work on Protozoa as regards the earlier period), it will be necessary to give a short account of the work done by later observers of this process, and more particularly of Keppen's work upon *Acineta papillifera*, the intrinsic merits of which have almost entirely been overlooked, owing largely, no doubt, to the fact that it was written in Russian. Keppen, in his paper published in the 'Memoires de la Societé des Naturalistes de la Nouvelle Russie,' Odessa, T. 13, 1888, states that he had not been able to follow the whole process of conjugation, of which he could only find some stages, but that the little he had seen had led him to believe that conjugation in this group, as in the Infusoria, was connected with a reformation of the nucleus, the new macronucleus being formed from the division of the micronucleus. He described in some detail the breaking down of the macronucleus, and in the later stages he saw amongst the degenerating masses of the old macronucleus lightly-staining bodies, which he regarded, on the analogy of Ciliata, as the division products of the micronuclei. Keppen figures four stages in conjugation, of which

fig. 50 shows the macronuclei drawn out into long strands and two micronuclear spindles, and fig. 31 shows an *Acineta* with a new light-staining nucleus surrounded by the small spherical products of the degeneration of the old nucleus.

At the time at which Keppen wrote the part played by the micronucleus in the conjugation of ciliates was not thoroughly understood, but it is to him we owe the first definite recognition of a micronuclear spindle in an acinetarian.

At the date at which Bütschli wrote his account of the Suctoria in 'Bronn's Thierreich' there were numerous isolated references to conjugation amongst Acinetaria to be found in the literature of this group. But as the only observers—Plate and Schneider—(with the exception of Keppen *vide supra*) who had followed the internal changes connected with this process, denied the existence of a micronucleus, it will be seen that much remained to be done. Bütschli himself remarks, p. 1917:—"Die vorstehenden Erwägungen zeigten, dass Copulationen nicht mit genügender Sicherheit erwiesen sind. Dagegen ist für die Dendrocometinen sicher, dass ihre conjugation im Wesentlichen wie die partielle der Ciliaten verläuft, woraus wohl geschlossen werden darf, dies gelten auch für die übrigen, nicht genauer untersuchten Conjugation."

In Maupas' paper, "Le Rajennissement Karyogamique chez les Cilies" (1889), he mentions without figures the results he had obtained in the conjugation of two acinetarians *Podophrya fixa* and *Podophrya cyclopus*. In both these forms there is only a single micronucleus, and in *Podophrya cyclopus* there is apparently complete fusion without the previous existence of any differentiation between the two gametes.

Maupas describes the conjugation of *Podophrya fixa*, which, as in the case I describe, is partial, in the following words (p. 385):—"Chez la *Podophrya fixa* j'ai observé les stades A, B, C and H Pendant ce dernier stade je n'ai jamais vu qu'un seul nouveau corps nucléaire et un seul micronucleus. Je ne serais donc pas étonné que la seconde

division de la nucleus mixte de copulation, correspondant au stade G, ne se produise pas chez cette espèce. L'ancien Noyau se desorganise et disparaît."

As regards the structure of the micronucleus, Maupas only states (p. 386) that it is "beaucoup plus tenu que chez les Ciliés, et sa substance se colore fort peu par les tinctures microchimiques. De là, avec d'autres causes qu'il serait trop long d'énumérer, ici les grandes difficultés de la mise en évidence. Elles sont, en effet, si grandes, que je considère l'étude d'une de les conjugations d'acinetiens, comme une des recherches les plus pénibles qu'un micrographe puisse entreprendre."

The next account of conjugation in *Acinetaria* is to be found in René Sand's 'Étude Monographique sur le Groupe des Infusoires Tentaculifères, 1901,' and I do not think that it can be regarded in any way as marking an advance in our knowledge of the process in this group, mainly, apparently, on account of the author's obsession by the theory that the *Acinetaria* are descended from the *Helioza*.

Sand considered that the micronucleus is a centrosome, and that the so-called conjugation is a plastogamy with two main aims (p. 101) :

(1) Une rénovation, un rajeunissement.

(2) Un processus de nutrition et de conservation ayant pour but d'égaliser, de 'moyenniser' leur situation nutritive.

His figures of the process do not show the nuclei at all, and apparently the whole of the observations, as apart from the conclusions which he formed, are contained in the following sentences (p. 99) :

"Nous avons toujours trouvé les deux individus placés sommet contre sommet, leur axes étant dans le prolongement l'un de l'autre ; les tentacules étaient rétractés ; les conjugaisons étaient très rares ; les deux individus, au stade observé, étaient séparés par une membrane, les noyaux étaient fragmentés et les cytoplasmas semblaient appelés à devoir se mélanger malgré la présence d'une membrane qui les séparait."

“Il était visible que la conjugaison avait lieu entre un individu riche, et un individu pauvre en tinctine.”

In 1902 Hickson and Wadsworth published a detailed account of the conjugation of what was at one time regarded as a very aberrant Suctorian *Dendrocometes paradoxus*, owing to the peculiar structure of the tentacular arms. They showed that the conjugation in this form was quite analogous to the process which occurred in the Ciliata. They found that there were normally three micronuclei, which undergo two successive divisions. Of the products of these divisions one divided again to form the male and female pronuclei. After cross-fertilisation the cleavage nucleus divides again twice in succession; there appears to be some doubt as to some of the later stages in the formation of the new nuclei, but normally two micronuclei degenerate; one becomes the new micronucleus, and the other develops into the macronucleus. In other cases the new macronucleus is formed by the fusion of two micronuclei, and in still other cases the three micronuclei divide again, the later stages in this form of evolution of the new nuclei not being followed. There are, however, some points in their paper, more especially as regards the part played by the macronucleus during this process, and also their general conclusions on the morphology of the cell body in Infusoria, to which it will be necessary to return after describing the conjugation of *Acineta papillifera*.

Methods. — As fixatives, Flemming's weak solution, Schaudinn's mixture, and corrosive-acetic were chiefly used, of which the weak Flemming solution seemed most suitable.

The preparations were stained either in alum-carminé or iron hæmatoxylin.

The material fixed in the osmic mixture was treated with picro-carminé before staining in alum-carminé.

ACINETA PAPILLIFERA (Keppen).—This species was first described by Keppen in his paper in the ‘Memoires de la Société des Naturalistes de la Nouvelle Russie Odesse’

(1888); he separated it from *Acineta tuberosa*, which it somewhat closely resembles owing to the presence of characteristic "valves" near the junction of the theca with the stalk, and to the fact that the body of the animal is usually not attached to the base of the theca. Keppen found it in both fresh and salt water in the neighbourhood of Odessa.

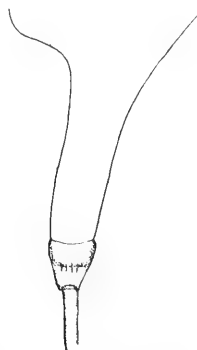
I first found this acinetarian in June, 1907, growing in great abundance on the hydrocaulus of *Cordylophora lacustris*, in Hickling Broad, the water of which is slightly brackish. It seemed to be feeding mainly on *Stentor*, which was at this time very abundant in the broad. As regards the main features of its structure, I have nothing material to add to Keppen's account, except as regards the relation of the theca to the body of the *Acineta*, and also as regards some details in the process of reproduction.

The "valves."—I always found three of these structures at the junction of the theca and the stalk, presenting under a low power an appearance corresponding with Keppen's figures, but in the interpretation of this appearance I should feel inclined to differ from Keppen.

Keppen regards these structures as three parallel plates cutting off the cavity of the stalk from that of the theca. I think that this appearance is merely the optical expression of a joint consisting of a short tube overlapping the ends of the stalk and of the theca.

These structures are unfortunately very difficult to make out in permanent preparations, but I arrived at this interpretation from an examination of the living animal before I had seen Keppen's paper, and it is, I think, confirmed by longitudinal sections (cf. text-figure 1). This interpretation would explain the frequent occurrence of individuals which have bent laterally at the junction of the stalk and the theca, so that the apical surface faces the base of the stalk. This bending is in some cases, I believe, purely passive, but I have often seen animals turn in this way by wrapping their tentacles around the stalk, and so pulling themselves

around. Cases in which either the stalk or the theca itself is bent are much more uncommon.



TEXT-FIGURE 1.—Section showing relation of theca and stalk in *Acineta papillifera*.

Cytoplasm.—The body of *Acineta papillifera* is prolonged anteriorly and laterally into two lobes from which the two bundles of tentacles arise. In the individuals from Hickling broad there were almost always two vacuoles lying side by side. Of these, one appeared to be a true contractile vacuole with a period of about one minute, whereas the other appeared to act as a reservoir, maintaining a constant size about a quarter of that of the full contractile vacuole.

In more or less starved forms the cytoplasm is quite hyaline, but in better fed forms the protoplasm may become very opaque owing to the presence of certain bodies with an affinity for nuclear stains. These bodies are apparently analogous with the Tinctin-körper of Plate VIII, the origin of which I have already dealt with in the case of *Tokophrya elongata*.

The macronucleus is generally an oval structure lying more or less centrally in the cytoplasm. Generally in whole stained preparations numerous spherical dark areas are to be seen resembling the so-called "Binnen-körper" of the Infusoria. In section these structures, as in the case of

some Infusoria (Bütschli, loc. cit., p. 1510) and Dendrocometes (Hickson), are found to consist merely of local thickenings in the mesh of the nuclear network, and therefore resemble karyosomes rather than true nucleoli.

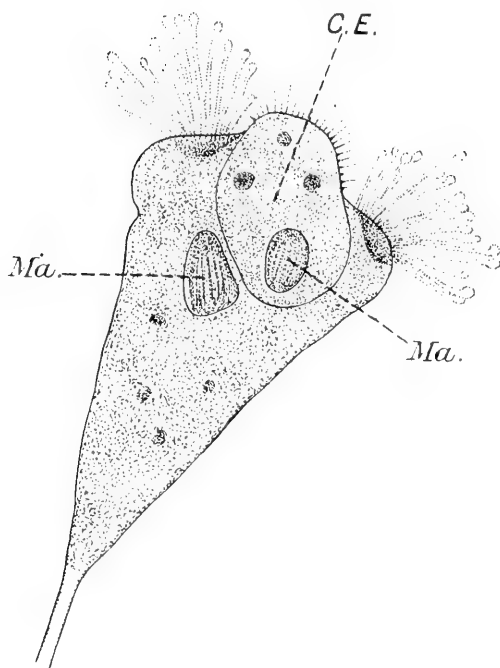
The micronucleus in the resting stage lies in a depression of the macronucleus, and consists of a membrane surrounding a clear area, in the centre of which lies a mass of feebly staining chromatin granules.

Reproduction.—As regards reproduction, the process which Keppen termed external budding is referred to at the end of the section upon conjugation. The only method of reproduction observed in the *Acineta papillifera* from Hickling was by the formation of single internal ciliated buds.

Keppen describes in addition to this for his form from Odessa, a process of multiple budding, but the figures that he gives are not convincing. One figure seems to me to show quite clearly that one so-called bud is simply a small individual with a fully developed stalk focussed through the large individual. The main features of the formation of the internal bud correspond with Bütschli's description of the formation of the internal buds in *Tokophrya quadripartita*. A small depression is formed on the apical surface of the acinetarian, which gradually widens so as to cut out a portion anterior to the macronucleus, the future bud.

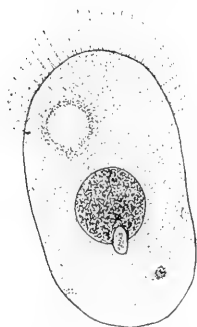
The free bud has a roughly cylindrical but slightly flattened shape (text-figs. 2 and 3). On its physiologically ventral surface it is covered with numerous rows of cilia. (Keppen speaks of 6—11 rows of cilia, but in the form from Hickling broad they seemed more numerous.)

There is a contractile vacuole on the left side near the anterior end, with a small reserve vacuole near it. The oval macronucleus lies near the centre of the body, and in favourable cases a micronucleus can be seen lying in a depression of the nuclear membrane. At first, after its escape, the embryo moves rather slowly, but a little later it moves rapidly through the water in characteristic sinuous curves.



TEXT-FIGURE 2.—Escaping ciliated bud of *A. papillifera*.
C.E. Ciliated bud. *Ma.* Macronucleus.

TEXT-FIG. 3.



TEXT-FIG. 4.

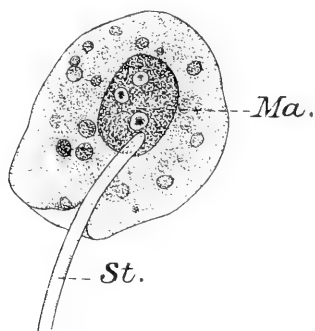


TEXT-FIGURE 3.—Free ciliated bud of *A. papillifera*. (Zeiss
 2 mm., apochr., comp. oc. 6.)

TEXT-FIGURE 4.—Later stage of free bud.

In an embryo (text-fig. 4) which left its mother at 10.45 p.m., the movements became very slow at about midnight, and it was fixed at 2 a.m. At this period the cilia had not yet disappeared, but the commencement of the stalk was already to be seen.

A slightly later stage of the development is seen in text-fig. 5, in which the stalk had almost attained its definitive length, and the cilia have disappeared. At a still later stage the tentacles make their appearance scattered irregularly



TEXT-FIGURE 5.—*A. papillifera* soon after attachment, showing disappearance of cilia and development of stalk. (Zeiss 2 mm. apochr., comp. oc. 6.) *Ma.* Macronucleus. *St.* Stalk.

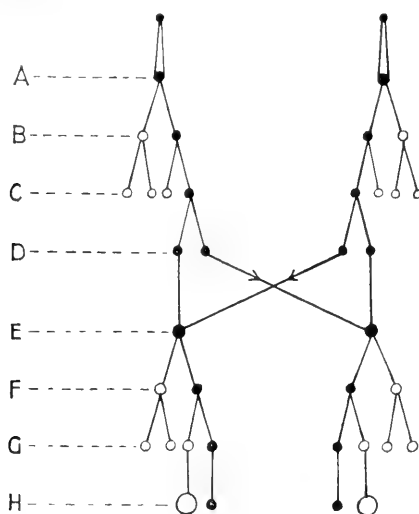
over the surface of the body, and the theca develops as a cup surrounding the body at its junction with the stalk.

Finally, the superfluous tentacles are withdrawn, and the animal becomes compressed laterally, assuming the shape of the fully developed form, the theca growing up to surround the whole body with the exception of a slit at the apical extremity, through which the lateral lobes bearing the tentacles protrude, and the ciliated embryos escape.

Conjugation.—As regards the early details of conjugation, there is no doubt that they agree with Maupas's general scheme for Ciliates (Scheme I).

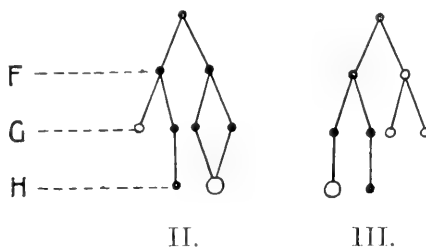
In preparations of the later stages the small size of the micronuclei, and the large number of chromatin-masses

scattered irregularly throughout the cytoplasm render it difficult to attain to absolute certainty as to the mode of



SCHEME I.—General scheme to illustrate the behaviour of the micronuclei of Ciliates in relation to conjugation—after Maupas. Micronuclei small black dots. Degenerating micronuclei small circles. New macronuclei large circles.

development of the new micro- and macro-nucleus. At first, I believed the normal process of reconstruction involved the



II.

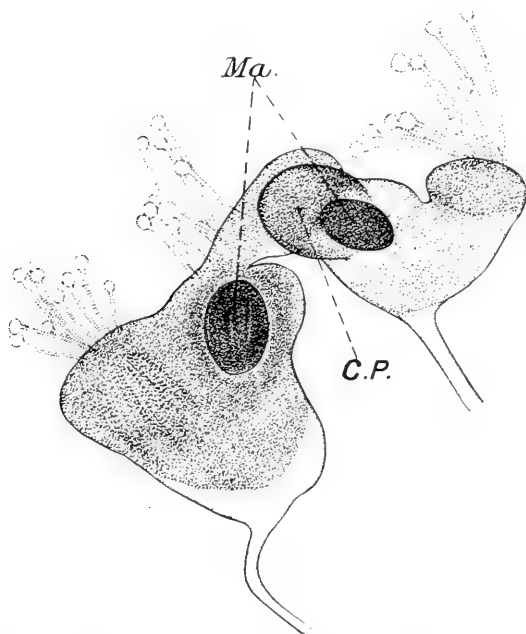
III.

SCHEMES II and III.—Diagrammatic schemes to illustrate the two possible modes of nuclear reconstruction after conjugation in *A. papillifera*.

growth of two of the four micronuclei into macronuclei, and their subsequent fusion (Scheme II). Later, however, I

became more inclined to the view that normally one micronucleus develops into the new macronucleus, one remains as the new micronucleus and two degenerate (Scheme III).

It is quite possible that both these methods of reconstruction occur normally since Prandtl has shown recently in a very careful work on conjugation in *Didinium nasutum*,



TEXT-FIGURE 6.—*A. papillifera*, first stage of conjugation, showing the prolongations (*C.P.*) by which contact between conjugating individuals is effected. *Ma.* Macronuclei. (Zeiss 2 mm. apochr., comp. oc. 6.)

that here the nuclear apparatus may be restored by one out of no less than five different methods, and Hickson has described three alternative methods in *Dendrocometes*.

The first indication of conjugation was found by Professor Minchin among specimens collected and examined on August 29th, and the majority of the individuals fixed at this period were found on examination to be early stages A—E. I have

no evidence as to the period occupied by the entire process of conjugation, but the pair figured in Pl. VII, fig. 8, which were found on fixation to have reached the final stage in the reconstruction of the nuclei, were followed for forty-six hours, but it is possible that this period is largely influenced by external conditions, e. g. temperature. Contact is usually effected between two conjugants by the prolongation of the apical lobes, the prolongations usually shortening as conjugation proceeds (text-fig. 6). More rarely the apical lobe of one conjugant may come in contact with the side of the other conjugant (Pl. VII, fig. 8), and a still more rare conjugation is purely lateral. In these latter cases the thin wall of the theca of one or both conjugants is dissolved away, so as to allow the contact between the cytoplasm of the two individuals.

Stage A.—The first internal indication of conjugation is afforded by the macronucleus. In Pl. VII, fig. 1, the macronucleus of the individual labelled A still shows the characteristic appearance of the resting nucleus with large darkly-staining areas, the pseudo-nucleoli. The micronucleus is unchanged, and closely applied to the macronucleus. The tentacles are partly withdrawn.

In B the macronucleus has already taken on the coarsely fibrillar appearance which is absolutely characteristic of the stages of its degeneration during conjugation. The micronucleus has left its original position near the macronucleus, and is preparing for the first division.

Stage B (Pl. VII, fig. 2).—The macronuclei of both individuals have become elongated, and the fibrillar appearance is more marked. The dividing micronucleus in the left-hand individual is covered by the macronucleus. The appearance of the micronucleus in the right-hand form seems to present some analogy to the so-called "Sichel" stage in the first division of the micronucleus in conjugating *Paramecia* described by Hertwig, and more recently by Hamburger.

Stage C.—Both individuals of the conjugation shown in

Pl. VII, fig. 3, contained two micronuclear spindles, which were slightly smaller than those of the first division.

Stage D.—This stage is, I believe, represented by Pl. VII, fig. 4. In each individual there is one micronuclear spindle near the line separating the two individuals, which would give rise to the male and female pronuclei. Of the other micronuclei, two in process of degeneration lie near the contractile vacuole, and the third is probably concealed by the macronucleus.

Stage E.—This stage is shown in Pl. VII, fig. 5, and the details of the male and female pronuclei under a slightly higher power in Pl. VII, fig. 6.

At this stage the degenerating macronuclei have become enormously drawn out, and as their length is so great, they are frequently thrown into coils which may abut on the partition dividing the conjugation, and readily give the appearance of a macronuclear conjugation. It is to such appearances as this that I think Hickson's account of macronuclear conjugation in *Dendrocometes paradoxus* must be attributed, and as Hickson seems inclined to attach much importance to this process (vide p. 349: "I am prepared, however, to go further than Sand, and regard the presence of the meganuclei in the conjugation processes not only as evidence of their relation to the interchange taking place in the cytoplasm, but as evidence of the necessity of the interchange of molecules of the substance of the meganucleus itself") it will be necessary to examine in some detail the evidence on which this meganuclear conjugation rests. This appears to me to be contained in the two following passages:

(1) Page 331. "At some time during the last three stages (H, J, K) the old meganucleus becomes very large, and is bent on itself in the form of a loop or horse-shoe. One extremity of this figure passes into the conjugative process, and approaching the limiting membrane, traverses it and fuses with the corresponding extremity of the meganucleus in the other individual. The exact phase at which this meganuclear conjugation takes place seems

to vary considerably ; all that can be said at present is that, as far as my experience goes, it usually occurs between stages I and K."

(2) Page 342. "In my preparations of Dendrocometes I have at least three cases in which the meganuclei actually touch, but a considerable number in which they approach one another very closely in the conjugation processes. That the junction is not merely casual contact, but actual organic connection, is proved by the preparation which is represented in Pl. 18, fig. 11. Here there is no sign of any boundary between the two nuclei, and the chromatin granules are fixed in such a manner as to suggest very forcibly that during life they were flowing from one side to another."

As at this stage of conjugation the macronuclei are rapidly degenerating, Hickson, in his attempt to show that this contact of the macronuclei is of fundamental importance in conjugation, puts forward the theory that "there is no inconsistency in the view that after the disappearance of the old meganucleus its nucleoplasm is still living in a modified form diffused through the cytoplasm."

The latter stages in the degeneration of the macronucleus in a conjugating acinetarian are almost precisely similar to the stages figured by Schaudinn in the digestion of the nucleus of a *Trichosphaerium sieboldi* which has been devoured by one of its congeners, and to the stages which I hope to describe in the digestion of the nucleus of *Stentor* by *Tokophrya*, and I believe that it is quite as justifiable to speak of the nucleoplasm still living in a modified form diffused through the cytoplasm in the one case as in the other.

I feel inclined, until further evidence is adduced, to regard the appearance figured by Hickson as abnormal, the forces which tend to elongate the macronuclei in the degenerating stages having carried the process too far, and breaking through the partition dividing the two conjugants led to the apparent macronuclear conjugation. In both the conjugants

in fig. 5 the remains of three degenerating micronuclei are to be seen.

In Pl. VII, fig. 6, the conjugating processes of the same pair are seen under a higher magnification. In the right-hand form the spindle of the fused male and female pronuclei is already formed. In the left-hand form the female pronucleus is lying close to the partition separating the two conjugants, whilst the male micronucleus is in a depression between the two conjugating forms.

This stage seems to be comparable with that figured by Hickson for *Dendrocometes*, Pl. 17, fig. 10, and shows that here, as in *Dendrocometes*, in contradistinction to the state of affairs found in *Paramecia*, the male and female pronuclei fuse not in the spindle, but in the resting stage.

It is also interesting to find that here, as in the case of conjugation in *Didinium nasutum* described by Prandtl, the male pronucleus seems to be considerably smaller than the female pronucleus.

The later stages in conjugation become exceedingly difficult to follow, as the chromatin of the macronucleus seems to be dissolved out of the achromatic network, and to be scattered through the cytoplasm in the form of darkly-staining, irregularly spherical blocks.

Normally, the zygote nucleus formed by the fusion of the male and female pronucleus appears to divide twice in succession, and of the products of this division two degenerate—one becoming the new micronucleus, and one the new macronucleus.

In Pl. VII, fig. 7, the achromatic network of the old macronucleus (*Ma.F.*) is still to be seen, though nearly all the chromatin appears to have been dissolved out, and to lie in irregular blocks (*Chr.*) in the cytoplasm.

Two of the division products of the zygote nucleus are to be seen as faintly staining vacuolar bodies, both closely applied to a mass of chromatin (*Mi.*). This relation suggests the absorption of the dissolved chromatin by the growing nucleus, a process to which Hamburger has drawn attention

in her recent account of conjugation in *Paramecium*. In this case probably the new macronucleus would have resulted from the fusion of these two bodies, the new micronucleus being probably covered by some part of the old macronucleus.

I can find no evidence in my preparations in support of Hickson's view that the feeble-staining capacity of the developing macronucleus is due to the fact that "at this stage either the whole or the greater part of the chromatin in its modified form passes into the surrounding cytoplasm, leaving the new meganucleus perfectly clear and homogeneous" (p. 345), a process which Hickson compares to the extrusion of chromatin in the egg cells of some Metazoa. I am more inclined to believe that this primary loss of staining power on the part of the developing macronucleus is to be explained by a simple increase of size, which is compensated for at a later stage by the absorption of the chromatin from the remains of the old macronucleus.

The final stage in the reformation of the nuclei is shown in Pl. VII, fig. 8. This pair of conjugants was examined at intervals from 10.45 p.m. on August 31st to 8.10 p.m. on September 2nd. Unfortunately, nothing could be made of the micronuclear changes in the living animal. During the last five hours the tentacles, which had been previously shortened, commenced to elongate, and when the individuals were killed they had almost reached their normal length.

From other observations on living material I am led to believe that the tentacles are not usually withdrawn during the early period of conjugation up to the stage F or G; during the much longer period associated with the final disappearance of the old macronucleus and the development of the new one the tentacles may become much shortened, and only attain their normal length after the reconstruction of the macronucleus, and shortly before the separation of the conjugants.

In the final stage the macronucleus has a very characteristic appearance of a lightly-staining loose mesh, with scattered

chromatin granules, and all traces of the old macronucleus have disappeared (Pl. VII, fig. 8).

External budding.—It will be now necessary to refer to a process which Keppen has termed “external budding,” and which he describes as the development of a lateral outgrowth, which may be followed by disappearance of the tentacles. He found that this outgrowth may change its shape and size considerably, and after a period of ten to twelve hours the animal may return to its original shape. In some very rare cases the outgrowth may break off, but he was never able to determine the fate of the bud so formed. In several cases he observed the nucleus carried to the boundary between the acinetarian and the so-called bud, and under these conditions he found appearances of fragmentation of the nucleus, though there was nothing to suggest a normal division.

In one case (fig. 47) he found a specimen of *Acineta papillifera* with a spade-like body containing a nucleus, and covered with moving cilia attached near the tentacles. This body, owing to its resemblance in shape, he was inclined to compare to the outgrowths described above. The body remained in contact with the acinetarian for some time, until they were finally both lost.

These structures are probably identical with those which Fraipont had previously described as “diverticules généra-teurs” in acinetan division, and which, according to him, were not to be regarded as external buds, but as structures out of which endogenous ciliated buds were to be developed. This theory was based on a single observation in which he found a ciliated bud in contact with a fixed form.

I believe that Keppen has confounded under this term two quite distinct phenomena—

(1) The formation of long conjugation processes in individuals which by reason of their position cannot come into contact with another individual ripe for conjugation.

(2) Cases of conjugation between a fixed form and a free-swimming ciliated embryo.

(1) It is interesting to notice that both Fraipont and Keppen examined individuals during a conjugation epidemic.

During the conjugation epidemic which I had the opportunity of observing I found, especially in material fixed during the commencement of the period, numerous examples of these long processes. The early stages of their formation are analogous to those of the formation of the conjugating processes, as may be seen by a comparison of text-fig. 6 and Pl. VII, fig. 9. I was never able to see an instance in which one of these so-called buds became free, but in several examples from later material I have found evidence of change in the nucleus (Pl. VII, fig. 9).

Although I have not been able to follow out these stages in detail, the appearance of the macronucleus is absolutely identical with the fibrillar stage which occurs previous to the fragmentation in normal conjugation.

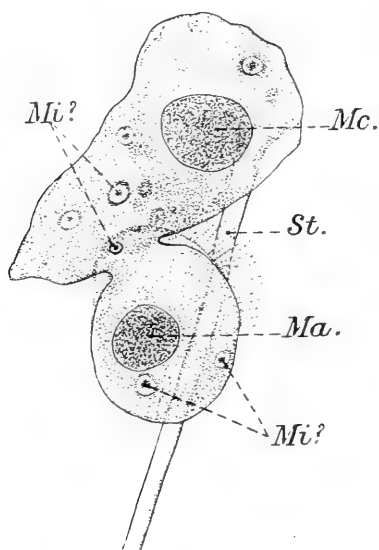
I am inclined to believe that under certain circumstances, e.g. the absence of mature neighbours within range, a process of parthenogenesis occurs similar to that described by Hertwig for *Paramecium aurelia* (p. 224), and by means of which Prandtl later has explained the behaviour of the third individual in cases of triple conjugation in *Didinium*.

It would seem that the chances against two neighbouring acinetaria exhibiting at the same time the three generally accepted symptoms of conjugation, viz. (1) sexual maturity, (2) distant relationship, (3) starvation, would be far more remote than the chance of meeting of two free swimming infusoria. And accordingly it is not surprising that in preparations made during a conjugation epidemic such appearances of presumable parthenogenesis are fairly frequent.

(2) Conjugation between a fixed individual and a free ciliated bud.

It is under this heading that I feel inclined to place Keppen's figure (49). I believe that this process is rare,

and, in fact, I was only once able (text-fig. 7) to follow the process in the living individual; but if the cilia, as is the case in the normal bud, disappear at the end of a couple of hours, it might be very difficult to distinguish the later stages of process (2) from those of process (1) (text-figs. 6 and 7).



TEXT-FIGURE 7.—A. papillifera, conjugation between a free ciliated bud and a fixed form. *Ma.*, *Mc.* Macronucleus. *Mi.* ? Micronucleus. *St.* Stalk. (Zeiss 2 mm. apochr., comp. oc. 6.)

IV. CONCLUSIONS.

(1) The Tintin-körper of *Acinetaria* are generally fragments of the ingested nucleus of their prey.

(2) Conjugation in *Acineta papillifera* agrees in all essentials with the process occurring in the Ciliate *Infusoria*, and that described by Hickson for *Dendrocometes paradoxus*.

It is possible that in those cases in which a fixed form cannot come into contact with another mature individual, a reorganisation of the nuclei may be effected associated with

either (a) conjugation with a free swimming ciliate bud, or (b) a process of parthenogenesis associated with the formation of the so-called "external buds" of Keppen.

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**Part II.—The Life Cycle of *Tachyblaston ephelotensis*
(Gen. et spec. nov.), with a possible identification of
Acinetopsis rara, Robin.**

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I. INTRODUCTORY.

In this part I wish to describe a new Acinetarian parasite—*Tachyblaston ephelotensis* (Nov. Gen. Nov. Spec.), which I found during a visit to Naples in May, 1908. I should like to take this opportunity of thanking the staff of the Aquarium for their kindness.

The early observers of parasitic Acinetaria regarded these animals as the embryos of their host, and it was not until 1860 that Balbiani put forward the view that the so-called embryos were really parasites. The truth of this theory was first shown by Metchnikoff in his early work on *Sphærophrya paramœciorum* in the 'Archiv für Anatomie und Physiologie' in 1864, to which he again refers in his classical work 'Leçons sur la Pathologie Comparée de l'Inflammation' (Paris, 1892). Metchnikoff showed that in this case the internal parasite divided, giving rise to tentacled buds, which, after developing cilia swam off and infected another host. It had frequently been suggested that certain rounded bodies found in Acinetaria were to be regarded as parasites, but this view has, as far as I am aware, never been confirmed, and Bütschli says in his account

of the Suctoria:—"Im Kapitel über die freie Knospung (s. p. 1894) wurde schon betont, dass gewisse angebliche Knospen einiger Arten möglicherweise kleine parasitische oder commensalistische Suctorien sind, welche auf grosseren leben. Ebenso fanden wir es nahezu, wenn nicht ganz gewiss, dass endosphaerenartige Suctorien in grosseren Arten ihres eigenen Stammes schmarotzen."

METHODS.—My material was fixed either with weak Flemming or corrosive acetic. Of these two methods the Flemming seemed to give far the most faithful cytoplasmic fixation. The Flemming preparations, after washing in water, were treated with a dilute solution of H_2O_2 in 70 per cent. alcohol, and then stained either in alum carmine or Mayer's hæmalum.

II. THE BUDDING OF EPHELOTA GEMMIPARA.

Before proceeding to give a detailed account of the somewhat complicated life-history of *Tachyblaston ephelotensis*, it will be necessary first to refer to the structure of the macronucleus and the mode of reproduction of its host *Ephelota gemmipara*, as it will only then become apparent how it is possible to distinguish the various stages in the life-history of the host from that of the parasite.

The macronucleus of *Ephelota* is generally described as a horse-shoe shaped structure lying in the horizontal plane of the animal, and giving rise to a varying number of branches, especially towards the apical surface, on which the buds are formed. The reproduction of *Ephelota* (*Podophrya*) *gemmipara* has been described by Richard Hertwig, whose results have been confirmed in all essentials by the later workers upon this form. The buds first make their appearance as small swellings round the apical pole, varying in number according to the size of the budding individual from 1 to 12. These small swellings slowly enlarge until they become elliptical bodies flattened along one surface, the

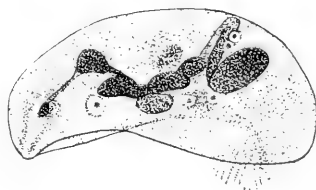
future ventral surface along which, at a later stage, a deep ciliated groove is formed. A branch of the macronucleus passes into each bud and becomes twisted upon itself in rather a complicated manner, so that the macronucleus of the young embryo at quite an early stage is already a twisted band (text-figs. 8, 9).

Shortly before the bud is set free the last strand connecting

TEXT-FIG 8.



TEXT-FIG. 9.



TEXT-FIGURE 8.—Normal *Ephelota* with external buds. (Zeiss 4 mm. apochr., comp. oc. 4.)

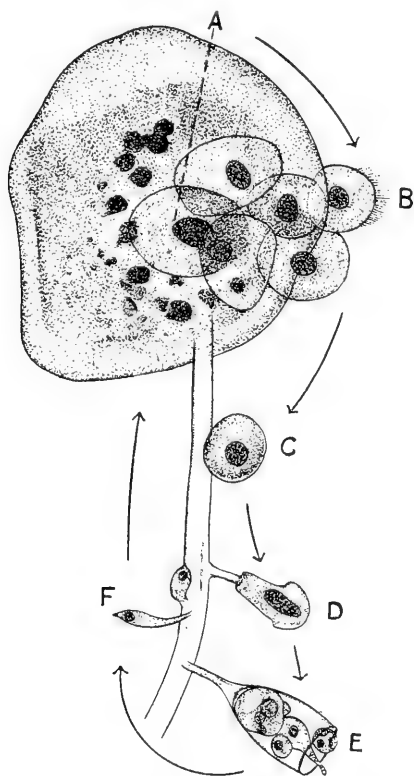
TEXT-FIGURE 9.—Free ciliated bud of *Ephelota*. (Zeiss 2 mm., apochr., comp. oc. 4.)

its macronucleus with that of the parent form is broken through.

III. TACHYBLASTON EPHELOTENSIS.

At the beginning of May received large quantities of brown seaweed (*Cystoseira*) from Nisida, which were simply covered by *Ephelota gemmipara*, visible to the naked eye as minute yellow dots. In material brought on the 6th May I was struck by the fact that some of the *Ephelota* contained peculiarly refractile rounded bodies, and that none of the *Ephelota* which presented this appearance

showed any trace of budding. Suspecting the presence of a parasite, I placed some of the infected forms in a deep watch-glass, and I was able to follow all the stages in the life cycle of the parasite in the living form.



TEXT-FIGURE 10.—Scheme of life-history of *Tachyblaston ephelotensis*. (Zeiss 4 mm. apochr., comp. oc. 4.) A. Internal parasitic form. B. Ciliated bud. C. Recently fixed ciliated bud. D. Later stage of fixed bud showing stalk. E. So-called "Acinetopsis" stage. F. Two free tentacled buds creeping up the stalk of the *Ephelota*.

In order to avoid recapitulation, a diagram of the life cycle of the parasite is shown in text-fig. 10. The internal parasite gives rise by division to ciliated spores. These, after a short

free life, fix and develop into a stalked form, which, on account of its great resemblance to a form previously described by Robin, may be termed the *Acinetopsis* stage. This gives rise, by repeated budding, to a large number of peculiar small buds, each of which is provided with a single thick tentacle. The tentacled buds become free, and crawl by means of their tentacle up the stalk of an *Ephelota*, which they infect.

In material brought on the succeeding days the ravages of the disease became more and more apparent, until in material collected on May 9th there was nothing to be seen except a large number of bare stalks of *Ephelota*, a few encysted forms, and a large number of the empty thecae of the parasite.

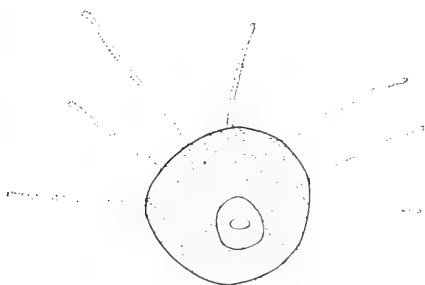
In material brought on May 12th I found, after much search, a few encysted *Ephelota* and some young healthy budding forms; these rapidly increased in number until the collected weed was again covered with *Ephelota*. On May 16th a second epidemic, which corresponded with the one described, recurred. I propose to call this parasite *Tachyblaston ephelotensis* in order to emphasise the extraordinary rapidity with which it infects large numbers of *Ephelota*.

THE INTERNAL STAGE OF THE PARASITE.—The infected *Ephelota* are readily distinguished even under a low power binocular microscope (1) by the absence of external buds, (2) by the presence of peculiar rounded bodies in their cytoplasm. In a fairly early stage of infection, shown in text-fig. 11, which was drawn from a living specimen, there was a single rounded body occupying the centre of the body of the *Ephelota*. The nucleus of the parasite could be clearly seen as light area lying in the cytoplasm. The cytoplasm in this, as in all the other stages of the parasite, is clearly marked off from the cytoplasm of its host, by the peculiar small refringent granules which it contains (Pl. VIII, fig. 5).

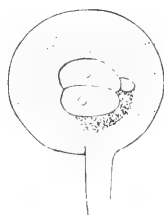
At first I was inclined to attribute this appearance to the presence of fat, which is very common in *Acinetaria*, but as

I could never find any trace of blackening after treatment with osmic acid in preparations in which the host showed considerable blackening this view had to be abandoned; on the other hand, in sections stained with hæmatoxylic eosin the granules seem to show considerable affinity for the eosin stain. I am inclined to regard the granules as reserve food stuff, as their number seemed to diminish in starved forms.

TEXT-FIG. 11.



TEXT-FIG. 12.



TEXT-FIGURE 11.—Early stage of infection of *Ephelota* by *Tachyblaston*, from the living animal. (Zeiss 16 mm. apochr., comp. oc. 8.)

TEXT-FIGURE 12.—Stage three hours later than that shown in text-fig. 11. The tentacles of the host are not shown. (Zeiss 16 mm. apochr., comp. oc. 8.)

About three hours later (text-fig. 12) the parasite had undergone one equal division and showed traces of the formation of a secondary bud. It seemed, as a result of examining a large number of preparations, that the first division is always approximately equal, but that this is followed by the formation of a number of true buds which do not obtain their full size at once. By the next morning seven internal parasites, some of which were developing cilia, could be counted in the above-mentioned specimen, and the cytoplasm of the host had become reduced to a thin shell surrounding the central mass of the parasites. As the budding of the parasite proceeds (Pl. VIII, fig. 6), frequently two or three chains of buds

are formed, radiating from a central mass, and each terminating in a ciliated bud. The nucleus in the internal parasite is a rounded homogeneous mass, which can be readily distinguished in stained preparations from the branched coarsely granular nucleus of its host. Under very high powers in material fixed with Flemming's solution it could be seen that the nucleus of the internal parasite is also very finely granular.

The ciliated spores (Pl. VIII, fig. 7) are more or less oval bodies, about $\cdot 04$ mm. long by $\cdot 018$ mm. broad, with a patch of long cilia on the right anterior area of the ventral surface. I saw no trace of the contractile vacuole in the living forms, but in most stained preparations a clear vacuole is present. The ciliated spores after a short free existence (about ten to fifteen minutes) settle down and begin to form a stalk (Pl. VIII, fig. 8). At this stage the whole animal becomes surrounded by a wall, the first trace of the theca, which is present, though not in so dense a condition, at a slightly later stage, even over the apical surface. In the fully developed stalked form (Pl. VIII, fig. 9) the theca measures from $\cdot 043$ to $\cdot 03$ mm. long by $\cdot 03$ mm. broad. The stalk measures from $\cdot 02$ to $\cdot 03$ mm. long, and is usually curved. In this stage the animal resembles very closely a form which was described many years ago by Robin, and which has not been seen since, *Acinetopsis rara*. Robin, in his account of this form, states that he only saw it three or four times in the midst of *Acinetaria* on stalks of *Sertularia*. He describes it in the following words :

"C'est un animal long de $0\cdot 07$ mm.— $0\cdot 09$ d'un tiers moins large, remplissant une coque ou theque en forme de verre à pied qui supporte un très grêle pedoncle long d'un dixième de millimètre.

"Corps uniformement grenu, grisâtre, avec une petite vesicule pulsatile, à surface libre plane, portant à son centre un tentacule et rétractile alternativement."

Robin himself stated that he knew nothing of the reproduction of this form, and later observers seem to have been

equally unfortunate, since Sand, in his monograph on the Acinetaria, says, "La classification de ce genre est provisoire, la mode de nutrition et de reproduction et n'ayant pas été observé."

The resemblance of this animal to the fixed stage of *Tachyblaston* is very striking, and this resemblance becomes even more significant when Robin's account of the formation of a second kind of small bud in *Ephelota* is taken into consideration. Robin did not follow the relation between the nuclei of these buds and the *Ephelota*, and Bütschli (p. 1894) refused to admit that these structures could really be regarded as buds of the *Ephelota*. From the consideration of these points, I think it very probable that Robin's "*Acinetopsis rara*" is merely a stage in the life-history of *Tachyblaston ephelotensis*. After the development of the theca the *Tachyblaston* starts budding very rapidly, the number of buds reaching twelve or fourteen. Each bud is provided with a single thick tentacle, the size of which, in comparison with other Acinetaria, seems out of all proportion to the size of the body. The bud, in this fully-formed condition with a contracted tentacle, has rather an elongated pear shape, the stalk of the pear being formed by the tentacle. In the centre of the body there is a large vacuolated nucleus; at the opposite pole to the tentacle the bud is produced into a curious short tuft, which I am inclined to regard as an adhesive organ. (This bud seems to bear a curious superficial resemblance to the genus *Rhynceta*, described by Zenker, from the appendages of a fresh-water Cyclops [Pl. VIII, fig. 10]). If the living buds are watched carefully it will be found that some of them shoot out their tentacles, which, at the same time, become very thin, to a length fully equal to that of the stalk and theca, about .07 mm. The tentacle then sways to and fro in a way that recalls the tentacle of *Urnula*. If the tentacle comes in contact with a foreign body, e. g. the stalk of an *Ephelota*, it retracts, and the bud is pulled out of its theca. Very frequently the bud remains for some time attached to its tentacle, swaying in a curious pendulum-like manner.

The body of the bud exhibits curious englenoid changes of shape, and in this way, with the aid of its tentacle, the bud can travel considerable distances up the stalk of the *Ephelota* which it is about to infect. After penetrating its host the tentacle seems to be completely withdrawn, and the bud seems to grow very rapidly to reach the size characteristic of the internal parasitic form (about $\cdot 04$ long by $\cdot 03$ broad), the life-cycle being thus completed.

The Effect of the Parasite upon the Host.—Metchnikoff, in his 'Lectures on the Comparative Pathology of Inflammation' (p. 27), has already put forward the view as regards *Sphærophrya parameciorum*, that "Pour se maintenir dans l'intérieur du protoplasma des infusoires les acinétiens doivent exercer quelque influence paralysante sur l'action digestive. Il est probable que ces parasites sécrètent quelque substance toxique, parce que'on a vu souvent divers infusoires tomber dans un état de paralysie et mourir à la suite des attaques des acinétiens libres. En végétant dans l'intérieur des infusoires, les acinétiens parasitiques provoquent une dégénérescence du noyau, qui se fragmente en grains ronds."

As far as I could see from sections of infected *Ephelota*, the effect of the parasite was far more marked upon the cytoplasm than on the nucleus of its host, and it is only in later stages of infection, when the cytoplasm has been reduced to a thin shell surrounding the mass of the parasites, that one finds traces of degeneration in the nucleus, the granules of chromatin running together to form darkly-staining lumps. It is, I think, a fact of some importance that here, where there is no direct absorption of the chromatin of its prey, the parasitic *Acinetarian* is quite free from so-called "tinctin-körper" of Plate.

Systematic Position.—From the account given of the adult structure of the *Tachyblaston* it must, I think, be regarded as closely related to the family *Urnulina*, and not as in any way related to the parasitic *Sphærophrya* described by

Metchnikoff.¹ It is also noteworthy that we have here, as in the case of many other parasitic animals, e. g. *Montstrilla*, a form the systematic position of which is quite indeterminate from the parasitic stages of its development, but which shows quite characteristic features in its later free-living stages. *Tachyblaston ephelotensis* is also of interest as showing the beginning of a development of a complicated life cycle in association with a parasitic method of life in a group of Protozoa which are characteristically free living, or, at any rate, purely external parasites. Starting from the *Acinetopsis* form, we have free-living tentacle buds, which, passing into their host, give rise, by a process of division, to ciliated spores of large size, which in turn develop on fixation into the *Acinetopsis* form from which we started.

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¹ The only other parasitic form which has been placed amongst the Acinetaria is that curious animal *Amoebophrya* which occurs in certain Radiolaria. I have never been able to understand from the published accounts of this animal why it was ever placed in this group; and in a recent paper by Neresheimer in the ‘Zoologisches Centralblatt’ it is far more appropriately placed amongst the Mesozoa.

EXPLANATION OF PLATES VII AND VIII,

Illustrating Mr. C. H. Martin's "Observations on Acinetaria."

PLATE VII.

Acineta papillifera.

LETTERING.

Chr. Chromatin. *C.P.* Conjugating process. *c.v.* Contractile vacuole. *Ma.* Macronucleus. *Ma.F.* Macronuclear framework. *Mi.* Micronucleus. *Mi.♂.* Male pronucleus. *Mi.♀.* Female pronucleus. *Mi.Sn.* Spindle of fused male and female pronuclei.

All figures, except 6, drawn under 6 comp. oc. + 2 mm. Zeiss apochr.

FIG. 1.—Conjugation stage A.

FIG. 2. " " B.

FIG. 3. " " C.

FIG. 4. " " D.

FIG. 5. " " E.

FIG. 6. " " E, detail.

FIG. 7. " " F.

FIG. 8. " " H.

FIG. 9.—So-called external bud.

PLATE VIII.

Tokophrya elongata.

FIG. 1.—Recently fed individual showing "Tintin-körper." (6 comp. oc. + 4 mm. apochr.)

FIG. 2.—Budding individual showing division of the macronucleus and "Tintin-körper." The tentacles were not drawn. (6 comp. oc. + 4 mm. apochr.)

FIG. 3.—Starved individual. (6 comp. oc. + 2 mm. apochr.)

FIG. 4.—Last stage of starvation. The pellicle is much wrinkled. The tentacles, and practically the whole of the cytoplasm, have disappeared. (6 comp. oc. + 2 mm. apochr.)

Tachyblaston ephelotensis.

FIG. 5.—Early stage of infection, showing a single *Tachyblaston* (*Ta.*) lying in an *Ephelota*.

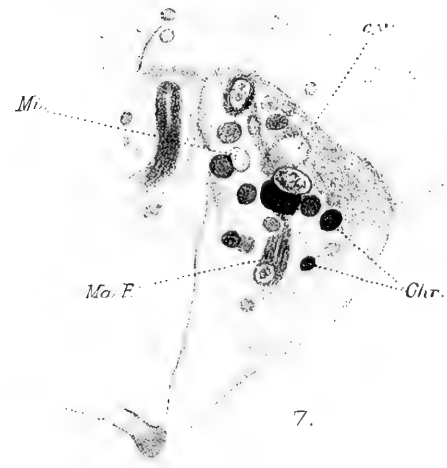
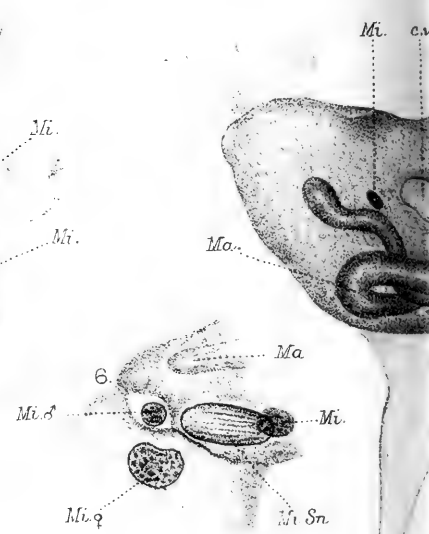
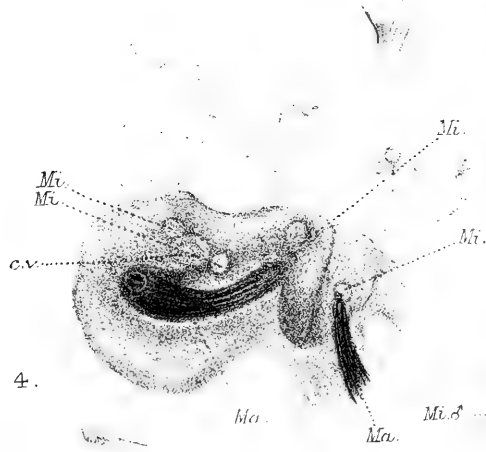
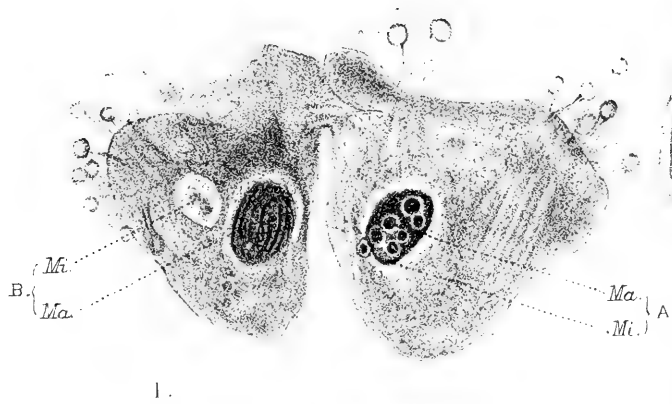
FIG. 6.—Late stage of infection, showing the formation of ciliated buds of *Tachyblaston*.

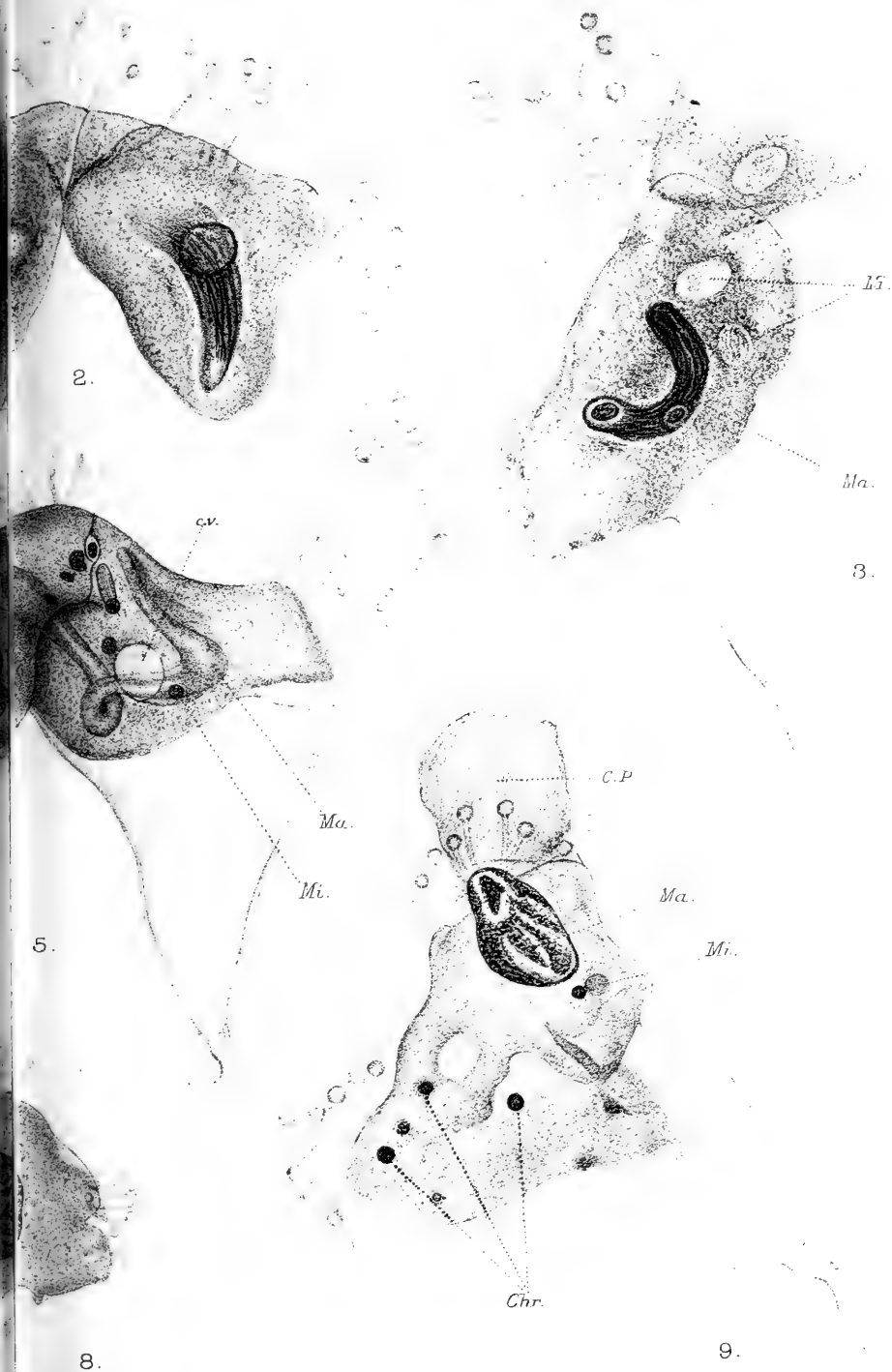
FIG. 7.—Free ciliated bud of *Tachyblaston*. Ventral view.

FIG. 8.—Recently fixed ciliated bud.

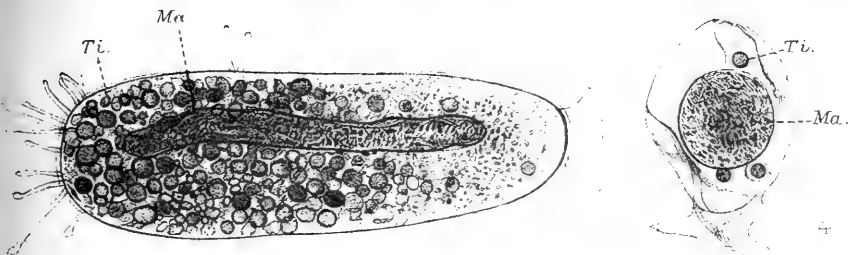
FIG. 9.—So-called "Acinetopsis" stage.

FIG. 10.—Wandering tentacled bud. "Rhynceta stage." (4 comp. oc. + 2 mm. apochr.)

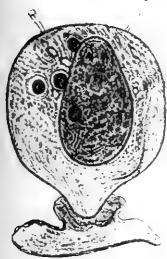




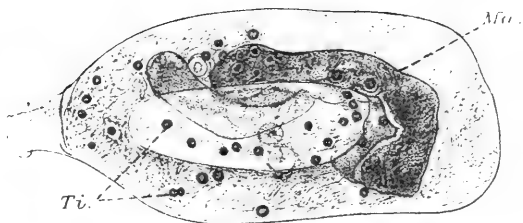




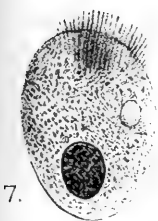
1.



3.



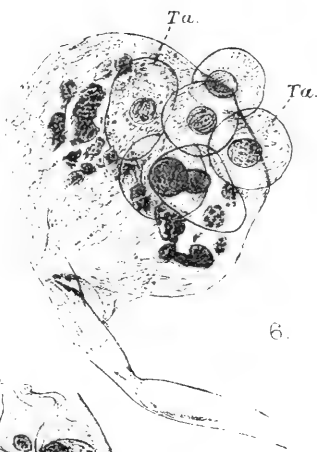
2.



7.



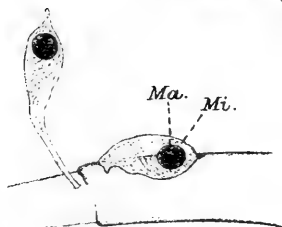
5.



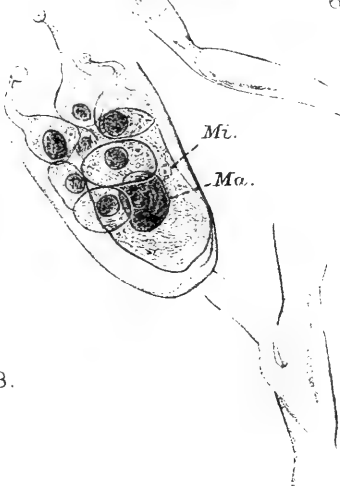
6.



8.



10.



9.

Studies on the Structure and Classification of the Digenetic Trematodes.

By

William Nicoll, M.A., D.Sc.,

of the University of St. Andrews.

With Plates 9, 10.

THE following work was commenced about the middle of 1907 at the Gatty Marine Laboratory, St. Andrews, under the Carnegie Trust Research Scheme. The greater part of it was done privately in the Pathological Laboratory, University College, Dundee, and it was concluded at the Marine Biological Laboratory, Millport, under a Government (Royal Society) Grant.

My best thanks are due to very many friends for assistance in various directions, and in particular to Professor McIntosh, of St. Andrews, for continual encouragement and suggestions, and to Professor Sutherland, of University College, Dundee, for granting me the privilege of working in his laboratory and for helping to elucidate several pathological difficulties.

In this paper an attempt has been made to allocate each of the forms dealt with to its approximate systematic place—a matter of considerable difficulty in many cases. At the present time a classification of the digenetic Trematodes in the true sense of the term can hardly be said to exist. To attempt to define a higher systematic unit than the genus is attended with much risk. A considerable number of sub-families have certainly been created, but few of these consist of more than two or three genera, and are really at most to be considered as indications according to which classification may be expected to proceed. The formation, however, of

these groups or sub-families and the examination of their relationships seems to be of the greatest importance in leading up to the natural family groups. On that account I have endeavoured—not in every case with success—to include each of the genera described here in some sub-family group.

Many systematic difficulties have been encountered in the course of this work, and I should like here to refer to the case of *Campula oblonga* Cobbold. It is almost a certainty that the form I am describing as *Brachycladium oblongum* (Brn.) is actually identical with Cobbold's species, but according to the strict laws of nomenclature the name not only of Cobbold's genus but also of his species must be regarded as *nomen nudum*. I have followed in this matter the authority of Looss and Odhner, but it seems to me that this particular species has been submitted to a test which, were it to be applied as rigorously, would prove fatal to many of the species and genera of older writers. There appears to be no escape from the trammels of nomenclature, and the only hope of simplifying matters consists in the submittal of each new or re-described species or other systematic unit to as strict a scrutiny as has been done in the case of *Campula oblonga*. This can never be done effectually so long as the work of criticism remains in the hands of isolated individuals. There is undoubtedly a need for some central scheme, as has been already advocated more than once.

The general anatomy and histology of the Trematoda has been the subject of fairly exhaustive research, yet a few points still remain doubtful and disputable, e.g. the function of the so-called subcutaneous glands and the large cells of the suckers. The myoblastic nature of these cells, as demonstrated by Bettendorf, can hardly be said to have been conclusively proved, or at any rate some modification and extension of Bettendorf's original views is necessary. The large cells of the suckers appear to have little in common with the subcutaneous cells. In appearance and staining properties they resemble true nerve-ganglion cells. The subcutaneous cells bear a much closer resemblance to the small cells of the suckers.

Few new results of a general nature are offered in the following notes. On the other hand, there are many apparent redundancies. These are only to be excused on the ground that the tendency to increased differentiation of specific forms, together with the fact that in numerous cases a form previously considered as a single species has on careful study of its anatomy and histology been subdivided into two or more distinct species, renders it necessary to give as full a description of the form dealt with as possible. In this connection reference to a much discussed paper of Stafford's¹ can hardly be omitted. Looss² has already expressed what must be the opinion of every systematist on this paper. Such a method as Stafford has employed is unpardonable even in a "preliminary contribution," and can hardly fail to cause endless trouble and confusion. By the laws of nomenclature Stafford's generic names, except where preoccupied, must be regarded as valid, for he is presumed to have in his possession type-specimens of the several genera which he has named, or at any rate type-specimens must be supposed to exist somewhere.³

For this reason Stafford's generic name *Fellodistomum* has been adopted here, although strictly speaking the name *Fellodistomum* is really synonymous with *Leioderma* Staff. (and therefore with *Steringophorus* Odhn.), for there is absolutely no difference of generic importance in Stafford's definitions of the two genera. His definition of *Fellodistomum* is actually a recapitulation of his definition of the preceding genus *Leioderma*, and the only difference is summed up in the sentence, "Many resemblances to *Leioderma*, but with sucker and genital glands crowded backwards." No mention is made of the position of the genital aperture, the absence of œsophagus, or the condition of the

¹ "Trematodes from Canadian Fishes," in 'Zool. Anzeig.,' xxvii (1904), pp. 481-495.

² "Revision of Hemiuridæ," in 'Zool. Anzeig.,' xxxi (1907), pp. 587-620.

³ Unfortunately this does not work out satisfactorily in every case. To my request (July, 1908) for type-specimens of several of his genera Dr. Stafford has vouchsafed no reply.

uterus and ova, which, as Odhner has in part indicated, and as I have tried to show, are really the generic features distinguishing *Fellodistomum* from *Steringophorus*.

Amongst the various histological features to which attention has been paid are the size of the muscle-fibres and of the elements of cellular structures. These, however, depending as they do so much on the state of contraction and the method of preservation, can hardly be considered of much practical utility. Other features of more importance which have been dealt with are the nature of the yolk-gland secretion (see under *Stephanophiala laureata*), the intestinal epithelium, and the cellular elements of the suckers in *Fellodistomum fellis*.

Reference may be made here to an interesting bionomic problem, which unfortunately does not admit of easy solution, namely, the length of time required for the cercaria to attain maturity on entering its final host. It is easily ascertainable that it does not commence to produce ova, which may be regarded as the criterion of maturity, immediately on entering its host. A longer or shorter period of time usually elapses, during which it increases considerably in size. The question has occurred to me in connection with various species, and here particularly in the case of *Lebouria idonea*. It has been a matter of frequent observation that a certain definite size limit exists between immature and mature specimens, or, in other words, that egg-production begins at a more or less constant period. In the case of *Lebouria idonea* the smallest mature specimen measured 1.6 mm. and the largest immature specimen was of the same length. Again, in *Podocotyle atomon* the limit of maturity appears to lie somewhere between .9 mm. and 1.0 mm. Many other examples might be given, but these will serve as illustrations. The smallest individuals found in the above two cases measured respectively 1.0 and .65 mm. It is fairly certain, however, that the cercariæ in each case are somewhat smaller than the above minimum limits, but taking them at those lengths it is evident that the cercariæ must

increase in length by at least half before attaining sexual maturity. This, of course, does not hold good in every instance, for in some species the proportionate increase is smaller, while in others it is larger.¹

That Distomids, like other animals, have a fairly constant maturity-size might have been deduced from analogy. The production of ova as the test of maturity is open to obvious objections. It might be held that the animal is mature when the genitalia are developed, and that egg-production need not proceed immediately although the animal continues to grow. Against this must be put the fact of the constant line of demarcation in size between specimens with ova and those without, and further, that a Distomid of adult size without ova is a rarity. Admitting that such a line of demarcation does exist, and that it is constant within close limits of variation, we should here have what might possibly be a valuable aid to diagnosis between nearly related species. As an example might be taken the case of the species of the genus *Levinseniella*. I have previously described a mixture of two or more of these as *Spelotrema feriatum* n. sp. (see later). They were found in five different species of birds. In three of these the Distomids were fully mature, but in the other two, namely, *Hæmatopus* and *Vanellus*, they were quite immature, although of the same size, or even larger. I have examined several specimens of both these birds at different seasons of the year and found the parasites fairly frequently, but never in the mature state. The probability is that the latter specimens represent a species distinct from that occurring in *Ægialitis* and *Pelidna*.

In the determination of the identity of similar parasites occurring in different hosts certain additional factors may enter, and it is to these that at several times by various authors variations in a particular Distomid inhabiting more than one final host have been ascribed. Differences of less or greater degree in external and internal characters, amounting in

¹ Looss deals with the same matter in "Distomen unserer Fische und Frösche," 'Bibliotheca Zool.,' vi (1894), p. 240.

many cases, as easily proved by later research, to specific distinction, have been attributed to the different environmental conditions in different hosts. That the variation in such cases is to be explained by the environment is not at all certain, and it is very probable that on careful examination the same amount of variation would be found in specimens collected from the same host. Certain features are capable of varying within comparatively wide limits and can be measured with sufficient precision, e. g. the size of the ova and the extent of the yolk-glands. A case in point is that of *Podocotyle atomon* (Rud.), which occurs in a large number of different fish. In this species the ova were found to vary considerably in size, and a list was given¹ of the size of the ova in specimens from four different hosts, which apparently showed that there were different limits of variation in each case. These measurements included a fair number of examples, but not nearly sufficient to determine the utmost limits in each host. One thing the list does show, however, is that in no particular species of fish are the ova distinct in size; such a variation, otherwise, would be sufficient to indicate specific distinction. I have since found in a single specimen of *Gastræa spinachia* a species of *Podocotyle* with ova greatly exceeding those from the fish already referred to and yet not differing in other respects from *Podocotyle atomon*. It is difficult, on this account, to say what effect, if any, environment may have on Distomid parasites, or whether the observed variations are purely the result of fortuitous circumstances. The question appears worth solving, and I hope to be able to publish later some observations on the same species found in numerous fishes from the west coast of Scotland.

The following is a list of the species mentioned in this paper, with their hosts and habitat:

¹ "Entozoa of British Marine Fishes," in 'Annals and Mag. Nat. Hist.' (7) xix, p. 76.

Stephanophiala (n.g.) laureata (Zed.)	Salmo fario	Intestine.
Stephanophiala transmarina n. sp.	Salvelinus fontinalis	} Intestine.
	Salmo mykiss	
Brachycladium oblongum (Brn.)	Phocæna communis	Liver.
Brachycladium sp.	Phocæna communis	Liver.
Lebouria (n. g.) idonea n. sp.	Annarrichas lupus.	Intestine.
Lebouria obducta n. sp.	Bairdiella chrysura.	Intestine.
(Lebouria) tumidula (Rud.) ?	Labrus bergylta	Intestine.
Podocotyle atomon (Rud.)	Pleuronectes flesus.	Intestine.
= Dist. vitellosum Johnstone	Pleuronectes platessa.	
	Gadus virens.	
	Gadus pollachius.	
Cainocreadium (n.g.) labracis (Duj.)	Labrax lupus	Intestine.
Peracreadium (n. g.) genu (Rud.)	Labrus bergylta	Intestine.
Peracreadium commune (Olsson)	Labrus bergylta	Intestine.
(Lepocreadium) serospinosum sp.		
inq.	Leiostomus xanthurus	Intestine.
= Dist. globiporum Linton e.p.		
Zoogonus rubellus (Olsson)	Anarrichas lupus	Intestine.
Fellodistomum fellis (Olsson)	Anarrichas lupus	Gall-bladder
Fellodistomum agnotum n. sp.	Anarrichas lupus	Gall-bladder and duodenum.
Stringophorus cluthensis n. sp.	Pleuronectes microcephalus	Intestine.
Plagiorchis notabilis n. sp.	Anthus obscurus	} Intestine.
	Motacilla flava	
Spelotrema claviforme (Brandes)	Pelidna alpina	} Intestine, cæca and rectum.
	Ægialitis hiaticula	
	Anthus obscurus	
	Numenius arquata	
	Motacilla flava	
	Larus ridibundus	
Spelotrema simile Jägersk.	Larus ridibundus	
Spelotrema excellens Nicoll	Larus argentatus	Intestine and cæca.
Levinseniella brachysoma (Crepl.)	Pelidna alpina	} Intestine, cæca and rectum.
= Spelotrema feriatum mihi, e.p.	Totanus calidris	
	Ægialitis hiaticula	
Levinseniella sp.	Hæmatopus	} Intestine, cæca and rectum.
	ostralegus.	
= Spelotrema feriatum mihi, e.p.	Vanellus vanellus	
	Numenius arquata	
	Larus ridibundus	
Tocotrema jejunum Nicoll	Totanus calidris	Intestine.

<i>Cryptocotyle concava</i> (Crepl.)	Phalacrocorax	} Intestine, cæca and rectum.
	graculus.	
<i>Gymnophallus dapsilis</i> Nicoll	<i>Oidemia nigra</i>	} Bursa Fabricii.
	<i>Oidemia fusca</i>	
<i>Maritrema humile</i> Nicoll	<i>Totanus calidris</i>	Intestine.
<i>Maritrema lepidum</i> Nicoll	<i>Larus argentatus</i>	Intestine.
<i>Maritrema gratiosum</i> Nicoll	<i>Larus ridibundus</i>	} Intestine.
	<i>Pelidna alpina</i>	
	<i>Ægialitis hiaticula</i>	
	<i>Hæmatopus</i> ostralegus.	

Sub-family STEPHANOPHIALINÆ, n. sub-fam.

Genus *Stephanophiala*, n. g.

Stephanophiala laureata (Zeder). Pl. 9, figs. 1-5.

Distoma laureatum Zeder, 'Nacht. z. Naturg. d. Eingeweidew.,' p. 192; Rudolphi, 'Entoz. Hist.,' ii, p. 413; 'Synops.,' pp. 113 and 413; Dujardin, 'Hist. d. Helminth.,' p. 435; Olsson, 'Kgl. Sv. Vet. Akad. Handl.,' 1876, Nr. 1, p. 21, Pl. iv, figs. 52-54; ? Linton, 'Rept. U.S. Commiss. Fisheries for 1889-1891' (published 1893), p. 553, fig. 26-30.

Distoma farionis Müll. Blanchard, 'Mem. Soc. Zool. France,' iv, 1891, pp. 481-3, fig. 38.

Crepidostomum laureatum (Zed.) Braun, 'Annal. Hofmus. Wien.,' xv, pp. 231, 232; Looss, 'Zool. Jahrbüch. Abtheil. Syst.,' xvi, 1902, p. 452.

This fairly frequent parasite of the common trout (*Salmo fario*) has been known for many years, and it has been found by various observers, who have each contributed towards a knowledge of the species, but no connected account has appeared since Olsson's description in 1876. Although not entirely free from error, this description is fairly accurate, but it is not sufficiently detailed for modern requirements.

Blanchard completed the topography of the female genitalia, and Looss was the first to indicate the structure of the circum-oral papillæ. Several other important features remain undetermined. The systematic position of the species will be discussed after a general account of its anatomy has been given.

The distribution of this Trematode may not be confined to Europe, for Linton has described what appears to be the same or a closely related species from an American trout (*Salmo mykiss*). No difference of specific importance is to be found in Linton's description, but it is probable that the two forms are not quite identical (see p. 35).

My specimens were obtained in June, 1907, from two small trout captured in a tributary of the River Spey (North of Scotland). Each fish contained four or five examples of the parasite. In another two small trout, from a stream running into the River Tay (April, 1907) several immature specimens (fig. 5) were found. The intestine of one of these fish was absolutely crammed full of *Echinorhynchus angustatus* Rud. while only one Distome was present. This might afford a striking instance of what Hausmann¹ terms the mutual crowding-out of parasites. Still another trout, of large size but in poor condition, was obtained later from the River Tay, but it contained no Trematodes. Four small trout were examined in May, 1908. They were also from the basin of the River Tay, but they did not contain *Distomum laureatum*. A few specimens, however, of *Bunodera nodulosa* (Zed.) were found in them. Olsson found the parasite from April to August, and Linton in July and August. I have had no opportunity of ascertaining whether it was present during the other months of the year. The occurrence in April of immature specimens only is suggestive, but my material is obviously inadequate to admit of any conclusion with regard to the life-history.

¹ "Über Trematoden der Süßwasserfische," 'Revue Suisse Zool.' v. This, however, can only be regarded as an occasional occurrence except in particular instances.

The number of specimens occurring in one host is not very great, although Linton puts it at as many as one to two dozen. Olsson says he found many specimens, but it is not clear whether he means in each or altogether. Its frequency, however, is well established, and it is probably more often present than not, at any rate during the months of April to August.

The habitat is chiefly the middle reaches of the intestine, but I have found it, especially in the immature state, as far down as the rectum. The occurrence of young specimens at a lower level in the gut than the adults is characteristic of more than one species, and it is probable that the cysts traverse the whole length of the intestine before the cercariæ are set free. Olsson records it from the rectum and stomach of the trout, also from the pyloric appendages of *Thymallus vulgaris*. Linton found it in the rectum of *Salmo mykiss*.

It is easily discoverable in the intestinal contents, being moderately large and of a pale but distinctly reddish colour. Olsson says the colour is white, and the difference may be due to the fact that my specimens were not seen till twenty-four hours after the death of the host, at which time the parasites were still alive, but very sluggish. There is no very obvious reason, however, for believing that that would account for a change in colour. For the above reason little movement was observed in the parasite, but it was apparent that the neck or pre-acetabular part was much more mobile than the rest of the body. The shape is elongated oblong, tapering towards either extremity, but more or less rounded according to the state of contraction; flattened dorso-ventrally. The continuity of the anterior margin is interrupted by the projection of two or more of the circum-oral papillæ. The surface of the body was thrown into irregular rugæ, but that is commonly seen in Trematodes some time after the death of their host. The length of my mature specimens is fairly uniform—3-4 mm.; the immature specimens are in many cases less than 1 mm. Most observers are agreed in fixing

the limits in size at 2-4 mm., but Olsson found examples measuring as much as 6 mm. The greatest breadth occurs at, or just behind, the ventral sucker, and is approximately one quarter of the length. This proportion is fairly constant.

The suckers are well developed and muscular. The oral sucker is sub-terminal and round, but not quite globular. In a specimen of 3.5 mm. length (to which all the following measurements may be referred) its transverse diameter measures .31 mm., while its antero-posterior diameter is usually slightly greater. Its aperture is not exactly circular, being encroached on laterally, but to no great extent, by the part bearing the two ventral papillæ. The wall of the sucker is not of the same thickness throughout, the anterior dorsal part being much thicker than the remainder. Here the thickness is .093 mm.; the rest of the wall varied from .05 mm. to .065 mm. It presents a further peculiarity in that the thick anterior part is separated from the rest by a distinct constriction where the thickness is not more than .035-.04 mm. The appearance suggests that the thick anterior part is a secondary growth from the main part of the sucker. The histological structure of the sucker represents what must be regarded as the typical Distomid structure. Externally it is separated from the body parenchyma by a well-defined fibrous membrane. Its cavity is lined by a cuticular layer continuous with the body cuticle, but somewhat thinner. Beneath this is a thin fibrous membrane continuous with the external membrane. Between the external and internal membranes run the radial muscle-fibres arranged in groups, each containing from six to twelve fibres. The latter may attain a diameter of .0045 mm., but this naturally varies with the state of contraction and is usually not more than .002 mm. The groups are separated from each other by spaces in which lie one or more cells. The anterior swelling is also traversed by radial fibres more closely arranged, and radiating, fan-like, from the inner membrane to the outer. Their course is not straight but curved (fig. 2). Close beneath each membrane there is a series of circular muscle-fibres, arranged

singly, one fibre being situated between the ends of adjacent radial fibres. Their diameter is about $\cdot 0006$ – $\cdot 0013$ mm., but the fibres of the external layer are somewhat stouter than those of the internal layer. Two distinct varieties of cells are met with in the sucker. The most numerous are small, rounded cells, which stain uniformly purple with hæmalum-eosin, and their nuclei are differentiated only by their darker colour. They are situated for the most part closer to the external membrane than to the internal membrane. Several filaments are given off by each cell. The other variety of cell is distinguished by being much larger and by different staining reaction. The cell body is not well defined, but the nucleus is large, conspicuous and round or oval in shape. It stains light pink, with a clear zone externally and a more granular zone internally. Within it is a small round nucleolus, staining bright red. These are the myoblasts, and they lie in the spaces between the groups of muscle-fibres and usually midway between the outer and inner membranes of the sucker.

The circum-oral papillæ are, as Looss has already pointed out, outgrowths of the muscle substance of the sucker. They are six in number, two being distinctly ventral and four dorsal, and their bases are all situated on the same transverse plane. The ventral papillæ are closely opposed to the margin of the aperture of the sucker, and their bases, as above mentioned, occasionally give rise to two prominences projecting into the aperture; the other papillæ are situated equidistant from each other and from the ventral papillæ. The shape is depressed, tongue-like; the size varies according to the state of contraction. In a transverse section of their base the measurements are $\cdot 035$ – $\cdot 04$ by $\cdot 015$ – $\cdot 02$ mm., while the length is about $\cdot 03$ – $\cdot 04$ mm. The muscle-fibres of the papillæ are apparently finer than those of the sucker. They comprise both longitudinal and annular fibres. No myoblasts were observed in the substance of the papillæ, but at the base of each, situated in the sucker, there is usually at least one myoblast apparently in connection with the papilla. A

feature of difference between the dorsal and ventral papillæ is due to the fact that the dorsal wall of the sucker is not closely apposed to the dorsal body cuticle, but is separated from it by parenchymatous tissue. The dorsal papillæ in finding their way to the exterior have to pass through this layer, and thus around the base of each dorsal papilla a small swelling is formed by the parenchyma, and this swelling is absent in the case of the ventral papillæ, for at their origin the wall of the sucker is contiguous with the cuticle and there is no intervening tissue. Still another point is to be remarked. The ventral papillæ arise from the already noted anterior swelling of the sucker, but the dorsal papillæ arise from the main part of the sucker, immediately behind the swellings. This is indicated in fig. 2. This circumstance seems to necessitate a modification of the views of Braun and Looss on the origin of the papillæ. It can still be maintained that the anterior swelling, together with the papillæ, represent the almost undivided suctorial swelling in *Rhytidodes gelatinosus* (R.), but it is evident that in *Stephanophiala laureata* the process of transformation has gone much further than was supposed. Thus the division of the simple swelling has given rise, not only to distinct contractile papillæ, but four of these have been entirely separated from the original swelling and have been displaced backwards.

The ventral sucker is situated near the junction of the anterior and middle thirds of the body. It is transversely oval and nearly twice as large as the oral sucker. Its transverse diameter is .50 mm. and the longitudinal diameter .43 mm., i.e. the diameter is about one seventh of the length of the body. Its depth is .16 mm. and occupies nearly the whole thickness of the body. The wall has an average thickness of .057 mm. The histological structure is essentially the same as that of the oral sucker, the muscle-fibres being, if anything, slightly stouter and the myoblasts proportionately less numerous. The sucker as a whole does not project much above the surface of the body.

A word must be said here with regard to the dimensions of the suckers as stated by Olsson. Considerable discrepancy exists, which necessitates an attempt at explanation. The sizes given by Olsson are $\cdot 40\text{--}\cdot 52$ mm. for the oral sucker and $\cdot 63\text{--}\cdot 75$ mm. for the ventral sucker. These are evidently much in excess of my measurements and cannot apply to a specimen of 3–4 mm. in length. Olsson says they were measured from a "spec. adult," and by this he must understand a specimen of 5–6 mm. in length. On this supposition the oral sucker would be about one eleventh of the body length and the ventral sucker about one eighth. Moreover, on careful measurement of Olsson's figure I find that the suckers are respectively one eleventh and two fifteenths of the body length. According to Linton also the oral sucker is one eleventh and the ventral sucker one eighth. In view of this agreement it may be taken as a distinctive feature of the species that the oral sucker is one twelfth to one eleventh and the ventral sucker one eighth to one seventh of the body length.

The cuticle has a fairly uniform thickness of $\cdot 0025\text{--}\cdot 003$ mm.

The musculature of the body conforms to the usual type, with the three subcutaneous layers, circular, longitudinal, and diagonal, and the less organised parenchymatous fibres.

Close underneath the layer of diagonal muscle-fibres lies a layer of what were formerly regarded as cutaneous glands, but which are, according to Bettendorf,¹ the myoblasts of the subcutaneous muscle-fibres. So far as can be gathered, Bettendorf's views have not been completely confirmed, but they are probably correct. At any rate the glandular nature of these cells has never been proved, and from Bettendorf's work they appear to be in intimate connection with the subcutaneous muscle-fibres. These cells, as I have observed them in this species, stain deep blue with hæmalum-eosin and the nuclei are only differentiated by being denser. The cells are rounded in outline with several filaments, and in many cases they appear fused together in groups of two or three,

¹ 'Zool. Jahrbuch. Abth. Anat.,' x, 1897, p. 307.

so that the outlines of the individual cells are lost. These cells certainly do not resemble the large cells (myoblasts) of the suckers, the cell-substance being much denser. They bear more resemblance to the smaller cells of the suckers, but most of all to the circum-oesophageal and vaginal "gland-cells." The glandular nature of these latter cells is again a matter of hypothesis, and their undoubted resemblance to the "subcutaneous glands" strengthens Bettendorf's views as to their myoblastic nature. The same may be said with regard to the cells surrounding the terminal portion of the ductus ejaculatorius in the cirrus pouch. All these cells are easily differentiated from cells of which the glandular function is well established, i.e. prostate gland and shell gland, even by ordinary staining methods.

True cutaneous glands, however, do exist in this species, but they are restricted in extent. They occur in the anterior part of the body only, situated fairly deeply and scattered throughout the region from the pharynx to the intestinal bifurcation. Similar cells (cephalic glands) appear to exist in many other species, but their occurrence has not been universally confirmed. They are not very numerous, but their number could not be estimated. Their ducts can be easily demonstrated, as also the fact that a considerable number of them open around the oral sucker. They are somewhat pear-shaped or tear-shaped, with their narrow end directed forwards. They measure about $\cdot 036$ by $\cdot 019$ mm., and their contents consist of a homogeneous, finely granular material, staining light blue with hæmalum-eosin, in the midst of which is a large round nucleus, $\cdot 007$ mm. in diameter, with distinct nucleolus. The function of these glands is still a matter of opinion. According to Leuckhart and Looss their secretion exercises an irritative action on the tissues of the host, causing an increased flow of juice, and even, according to some authors, a flow of blood in certain cases. This seems a reasonable enough supposition and is supported by the position of the glands. It may also account for the fact that some animals, although harbouring an immense number of

Trematode parasites, do not appear to suffer from their presence, but may indeed possibly benefit by the increased stimulation of the gastric and intestinal glands. An alternative theory with some claim to recognition is that the secretion of these glands renders the contents of the host's intestine less tenacious and thereby facilitates the activity and locomotion of the parasite.

The alimentary system does not offer any striking peculiarity. There is a small pre-pharynx, with thin walls and lined by cuticle continuous with that lining the oral sucker and pharynx. Into this the anterior end of the pharynx projects, so that, on external view, the pharynx appears to be continuous with the oral sucker. In many species the pharynx is described as continuous with the oral sucker, but in most of those it will probably be found that a small pre-pharynx intervenes. One notable exception is *Rhytidodes gelatinosus* (Rud.), in which no pre-pharynx is present. The pre-pharyngeal vestibule gives the pharynx greater freedom of movement, permitting, as it does, a certain amount of longitudinal movement, independent of the body extension and contraction, while at the same time it allows the anterior end of the pharynx to expand, somewhat after the manner of a sucker. The external membrane of the oral sucker is continued along the pre-pharynx on to the pharynx, which it invests. The pharynx is of moderate size, and nearly globular, but flattened dorso-ventrally. Its diameter is .15-.19 mm. and thickness .09-.10 mm. The lumen is a narrow, transverse slit, and the walls are about .04-.05 mm. thick. The structure is identical with that of the suckers. The radial muscle-fibres have an average thickness of .0015 mm. and the circular fibres .001 mm. Towards the anterior end the muscle-fibres are very closely packed. Passing forwards the radial fibres are more and more obliquely set, until they come to run almost parallel to the internal wall. Lacunar spaces are much reduced and cells are rare. Most movement apparently takes place at this part.

The œsophagus is about the same length as the pharynx

(1.5–2 mm.) and the intestinal bifurcation occurs a short distance in front of the ventral sucker. The diverticula are uniform and fairly wide, extending almost to the posterior end of the body. The œsophagus is lined by a somewhat thick membrane (metamorphosed epithelium), the surface of which is thrown into slight folds. This membrane is continuous with the cuticular lining of the pharynx, but is thicker and stains differently. With hæmalum-eosin it takes on a reddish-purple colour, whereas the cuticle is more distinctly blue. The musculature consists of the usual internal annular and external longitudinal layer, the fibres being more or less widely separated according to the degree of contraction. Surrounding the outer layer is a ring of very loose connective tissue, on the outer edge of which are numerous small, blue-staining cells—the myoblasts of the œsophageal muscles. The diverticula separate at a wide angle and bend towards the outer side of the ventral sucker. The rest of their course is practically straight, with a slight turn in near the end. At first they lie midway between the dorsal and ventral surfaces of the body, but on passing the ventral sucker they assume an unvarying dorsal position and are surrounded on all sides except dorsally by the yolk-glands. The average width of each diverticulum is about 0.8 mm. Their transverse section appears sometimes oval, sometimes triangular, and sometimes almost circular. The lining consists of a single layer of epithelial cells, with well-marked nuclei and nucleoli. The nuclei measure 0.05–0.08 mm. The cells are of irregular shape, with long fibrillar or hair-like processes stretching into the lumen and filling it up to some extent. Amidst the cells there are numerous small vacuoles. The appearance presented, in fact, is that of a row of nuclei lying in the midst of a fibrous feltwork; the cell outlines are difficult to distinguish. This epithelial layer is absent in a small part of the diverticula just behind the bifurcation, and is replaced by a continuation of the œsophageal membrane. This recalls the condition in *Patagium brachydelphium* Heymann, in which the initial part of the diverticula is constricted and devoid of

epithelium. It is thus histologically part of the œsophagus. This condition is of some structural importance. In other species which I have been able to examine, and particularly in the ALLOCREADINÆ, the layer of epithelial cells is continued right up to the junction with the œsophagus. The musculature of the diverticula is the same as that of the œsophagus, but the fibres are more irregularly spaced.

The excretory vesicle consists of a single undivided terminal sac, lying just under the dorsal surface of the body and extending as far forwards as the anterior border of the anterior testis, where it divides into two much narrower tubules running forwards to the level of the oral sucker. Over the testes the vesicle is compressed transversely, but elsewhere it is nearly isodiametric. The excretory pore is not quite terminal but is displaced very slightly dorsally. In section the wall presents an irregular outline. The lining of the vesicle consists of a distinct but thin membrane, which stains like cuticle, and under this there is a regular layer of oval nuclei with their long axes parallel to the wall of the vesicle. No cell outline could be discriminated. The nuclei possess a distinctive character in the apparent absence of nucleoli. They contain numerous chromatophil threads and granules staining rather dark blue, while the rest of the nucleus has a lighter tint. These nuclei can be traced throughout the greater part of the excretory system and appear to be peculiar to it. This lining membrane evidently does not conform to the usual type, in which the epithelial cells are prominent in the wall of the vesicle and are sharply marked off from the surrounding tissues. Here the cells are not prominent, and they are in close relation to the adjoining parenchyma.

Genital system: The testes are two rounded or ovoid bodies, situated directly behind each other in the middle line of the body. The posterior testis is about one quarter of the body length from the posterior end, while the anterior testis is separated from the ventral sucker by a shorter space. They may be closely apposed to each other, or more usually

separated by a narrow space occupied by yolk-glands. Their outline is slightly irregular, but not lobed. The anterior testis is often hollowed out in front and transversely elongated; it varies in size and shape much more than the posterior testis does. The antero-posterior axis measures $\cdot 21$ – $\cdot 28$ mm., the transverse axis $\cdot 26$ – $\cdot 44$ mm. The corresponding measurements of the posterior testis are $\cdot 28$ – $\cdot 43$ mm. and $\cdot 29$ – $\cdot 39$ mm. The average is about $\cdot 27$ by $\cdot 34$ mm. for the anterior, and $\cdot 34$ by $\cdot 33$ mm. for the posterior. Thus the diameter is rather less than one tenth of the body length. This result is in close agreement with the figures of Olsson and Linton. Olsson, however, states that the testis exceeded the ventral sucker in size. That was certainly never the case in any of my specimens, nor does it appear to be so from Linton's figure.

There is no sinus genitalis, or at most it is represented merely by a shallow depression, in which the male and female ducts open separately but contiguously, the former in front and to the left of the latter. In total preparations, however, only one aperture is sometimes seen. Its position is invariably median and exactly ventral to the posterior end of the pharynx. In Olsson's representation of the species (Pl. IV, fig. 52) the apertures are shown widely separate, but this is evidently the result of an erroneous observation, which I shall endeavour to make clear. The apertures are figured behind the intestinal bifurcation, between it and the ventral sucker, and they appear to be separated by a distance of nearly $\cdot 2$ mm. Further, from the supposed male aperture a long penis is represented stretching straight forwards to about the middle of the pharynx, while the cirrus-pouch shows a somewhat elongated oval outline quite different from the pear shape of my specimens. To me it appears that the structures marked *p* and *b* in fig. 52 are really portions of the cirrus-pouch, *b* being the thicker posterior part and *p* the narrow anterior part or neck running forward to the genital aperture near the posterior end of the pharynx. The erroneous position of the female genital aperture is probably due to the fact that

Olsson mistook the point beyond which the ova could not be seen for the termination of the vagina—a not at all unlikely mistake. On such a supposition my specimens agree very well with those of Olsson, otherwise the difference is of more than specific value.¹ On this matter Linton says nothing definite, but his figures show the genital aperture well forward in the neck, always single and sometimes with exerted cirrus. Careful measurements of his drawings, which we may assume to be drawn in proportion, show that the genital aperture is nearer to the posterior end of the pharynx than to the anterior border of the ventral sucker. In fig. 30, where the pharynx is not represented, the genital aperture is about .19 mm. behind the posterior border of the oral sucker. The average length of the pharynx being .16 mm. it is evident that the genital aperture is not far behind its posterior border.

The fact that Linton represents the genital aperture single tends to cast some doubt on my observation of the absence of a sinus genitalis. The exerted cirrus in Linton's specimens might have obscured the double aperture, but it is possible that the condition as I observed it was purely accidental. Against this is the fact that it occurred in several specimens.

The cirrus-pouch is, as already mentioned, of the characteristic Distomid shape, with a wide posterior part containing the vesicula seminalis, gradually narrowing into a slender anterior part containing the terminal part of the ductus ejaculatorius. The latter usually passes straight back from the genital aperture, and the thicker part is bent on it either to the left or to the right. It extends back to the level of the centre of the ventral sucker. It occasionally does not

¹ By the kindness of Dr. Jägerskiöld I have been enabled to examine two of Olsson's original specimens from *Thymallus vulgaris* and to confirm the correctness of the above suppositions. The genital aperture (or closely apposed apertures) is situated midway between the two suckers and therefore not far behind the end of the pharynx. Its position is probably most correctly described as midway between the suckers.

reach this level, but seldom, if ever, exceeds it. Its length is $\cdot 45$ – $\cdot 55$ mm.; the maximum diameter is $\cdot 12$ mm., and the average diameter of the neck is $\cdot 05$ mm.

The pouch is a true muscular cirrus-pouch, and its wall consists of the frequently described internal annular layer and external longitudinal layer. It thus differs essentially from that of *Bunodera nodulosa* (Zed.), in which the wall is purely membranous.

The structures contained within the cirrus-pouch conform to the common type, e. g. that of the ALLOCREADINÆ, but they are simple and less complicated. The vesicula seminalis is an undivided elongated oval sac (fig. 3), measuring $\cdot 2$ by $\cdot 04$ mm. It is not in the slightest degree convoluted. It is surrounded on all sides by the cells of the prostate glands, except towards the posterior end, where it is contiguous with the wall of the cirrus-pouch. The length of the ductus between the vesicula and the pars prostatica is about $\cdot 15$ mm. For the first two thirds of that length it has a diameter of $\cdot 015$ mm.; it then somewhat suddenly widens to $\cdot 023$ mm., which diameter it retains till it passes into the pars prostatica. The latter has a length of $\cdot 05$ mm., and a maximum diameter of $\cdot 035$ mm. It has a globose shape, and into it open the ducts of the numerous prostatic cells. These are entirely confined to the part of the cirrus-pouch behind the pars prostatica, so that their ducts all pass forward. They are large tear-shaped cells elongated in the long axis of the cirrus-pouch, and having an average size of $\cdot 027$ by $\cdot 012$ mm. Their outline may be more or less quadrilateral owing to compression. They stain deep purple with hæmalum-eosin, and have distinct round, rather small ($\cdot 004$ – $\cdot 005$) nuclei. The terminal part of the ductus ejaculatorius, which, although not extended in any of my specimens, functions as an exsertile cirrus, is slightly convoluted. As a rule it describes only one turn, a little in front of the pars prostatica, but its position is not constant. The length of this part is about $\cdot 1$ mm. and its diameter is $\cdot 02$ – $\cdot 025$ mm. Between this part and the wall of the cirrus-pouch are numerous small, round cells.

These are obviously not prostatic cells, for they present a different appearance and take on a distinctly blue stain. Moreover, ducts cannot be made out. Various functions have been ascribed to these cells. Most frequently they have either not been noted or considered to be prostatic cells. They have also been regarded as glands opening into the ductus ejaculatorius. Neither of these theories has been verified. The staining reaction differentiates them from the prostate cells and from similar cells, e. g. those of the shell-gland. On the other hand, they bear a distinct resemblance to the circum-oesophageal and peri-vaginal cells, as also to the "subcutaneous gland cells" (i. e. myoblasts). The resemblance to the circum-oesophageal cells, which Bettendorf¹ has shown to be the myoblasts of the oesophageal muscle-fibres, suggests that the cells surrounding the ductus ejaculatorius, as also the peri-vaginal cells, are the myoblasts of the muscle-fibres of the walls of these structures.

The ovary is an almost globular body situated close behind the ventral sucker and a considerable distance in front of the anterior testis. It may lie on either side of the middle line, or, according to Olsson, it may be median. In none of my specimens did it occur in the latter position; it was most frequently on the left side. The size is .23 by .19 mm., the thickness .16 mm. The configuration of the shell-gland complex has already been carefully described by Blanchard. Just behind the ovary lies a small oval receptaculum seminis. From its inner side there arises a duct giving off Laurer's canal, which is a straight tube of moderate length, and then running forwards to join the oviduct. The latter issues from the inner surface of the ovary, and passes backwards. After being joined by the duct from the receptaculum seminis, it turns forwards to pass into the ootype, and thence into the uterus. Blanchard's representation of the shell-gland is somewhat diagrammatic. It is much more extensive and is not enclosed within a membrane. It lies under the dorsal

¹ "Über Muskulatur und Sinnezellen der Trematoden." *Zool. Jahrb. Anat.*, x (1897).

surface of the body, and consists of a large number of small cells closely aggregated and composing a fairly distinct body to external view.

The common yolk-duct joins the oviduct between the entrance of the ductus receptaculi and the ootype. The yolk reservoir lies just behind the shell-gland and is median. It forms a more definite swelling than Blanchard indicates. It is formed by the junction of the two transverse yolk-ducts. The longitudinal ducts run parallel to the intestinal diverticula and along their ventral surface. The yolk-glands consist of two lateral groups of follicles extending continuously from the level of the pharynx to the posterior end of the body. Behind the posterior testis they extend in towards the middle line and fill up the posterior part of the body. In addition offshoots are sent in between the testes and in front of the anterior testis, and these almost unite in the middle line, thus separating the testes from each other and from the uterus. There is no tendency towards proliferation in front of the ventral sucker, the lateral fringes having a fairly constant width. Their situation is particularly towards the ventral surface, so that laterally they lie ventral to the intestinal diverticula and posteriorly they do not approach the dorsal surface to any great extent. The follicles are irregularly ovoid and measure $\cdot 08$ – $\cdot 10$ by $\cdot 04$ – $\cdot 06$ mm. Each contains about a dozen gland cells, which present two distinct appearances. In each follicle there is almost invariably one cell situated centrally (fig. 4) differing from the surrounding cells in shape and staining reaction. This central cell appears to be free, not much compressed by the neighbouring cells, and in consequence has a regular oval outline. The remaining cells are closely pressed together into various polyhedral shapes. The staining reaction is more distinctive. The stain used was weak hæmalum with aqueous eosin, but corresponding results were obtained with combinations of hæmatoxylin, methylene blue, and saffranin. The peripheral cells take on a uniform purple colour, while the central cell appears pink with a circumferential granular zone. The nuclei

also differ. In the peripheral cells they appear to consist of three concentric layers. The outmost zone is granular and stains deep blue, the middle is white, while the central part, the nucleolus, stains bright red. In the central cell, on the other hand, the nucleus stains uniformly bright red and appears to consist of little more than the nucleolus. The cells measure $\cdot 02\text{--}\cdot 03$ mm. in diameter, while the nuclei measure $\cdot 006\text{--}\cdot 0075$ mm., but those of the peripheral cells appear to be slightly larger than that of the central cell. The central cell is obviously the most mature part of the follicle, and it is probable that only in this condition are the yolk-cells despatched from the follicles. The yolk-ducts and reservoir, at any rate, contain only such cells. In their passage down the yolk-ducts they are compressed into a cubical or short cylindrical shape; in the reservoir they become polyhedral, but they again become cubical on passing into the common yolk-duct. They retain their bright red nuclei throughout, and these are still conspicuous within the most mature ova. No intrinsic muscle-fibres or true epithelium of the yolk-follicles or yolk-ducts could be observed, but this does not prove their absence as they are particularly difficult to demonstrate.

Turning back from the ootype the uterus describes a moderate number of convolutions in the space between the posterior border of the ventral sucker and the anterior border of the anterior testis. Laterally it is confined by the intestinal diverticula. Owing to its thickness the ovary is not overlapped by the uterus. The convolutions tend towards and cause bulging of the ventral surface of the body. The terminal part of the uterus runs straight forward on the right side of the cirrus-pouch and passes into the vagina at the level of the posterior end of the pouch. The vagina is also straight and has the usual muscular structure. Its diameter is $\cdot 019$ mm. in the undilated condition. The cuticular lining is $\cdot 004$ mm. thick. The thickness of the combined muscular layers is $\cdot 003$ mm. and the diameter of the lumen is only $\cdot 005$ mm. To admit the passage of the ova it

must therefore be capable of considerable dilatation. It is surrounded by lax connective tissue, in which are embedded the peri-vaginal cells already referred to.

The ova are usually less than 100 in number. The shape is ovoid, slightly blunter at the opercular pole. The shell is .0018 mm. thick and bright yellow, not darkening much throughout the uterus. The size of the ova is .075-.080 by .040-.044 mm.; this is somewhat larger than the size quoted by Olsson, but agrees closely with that of Linton. There is absolutely no intra-uterine segmentation, the ovarian ovum remaining undivided till deposition. The yolk-cells within the egg also remain unchanged, and can be easily distinguished from the ovarian cell by their bright red nuclei, the nucleus of the ovarian cell taking on a darker, nearly purple colour with hæmalum-eosin.

Olsson's figure (Pl. IV, fig. 52) gives a somewhat erroneous impression of the appearance of the ova and uterus in a specimen of average size. The ova are usually proportionately larger and the convolutions of the uterus are indistinguishable.¹

Systematic position of *Distomum laureatum* Zeder.

The inclusion of this species within the sub-genus *Crosso-dera* by Dujardin in 1845 need not be discussed here. The most recent attempt to define its systematic position is that by Braun,² and his classification is accepted by Looss,³ Heymann,⁴ Pratt⁵ and Stafford⁶. Braun included it in a new genus, *Crepidostomum*, of which the type is *C. metæcus* (Bru.) from bats. The two species are certainly very nearly

¹ In the two specimens from Olsson's collection which I examined the uterus does not have the disposition represented by Olsson. The ova are congregated more over the dorsal surface of the ventral sucker, so that they are best seen on viewing the animal from the dorsal surface.

² 'Annal Hofmuseum, Wien,' xv (1900), p. 232.

³ 'Zool. Jahrb. Abth. Syst.,' xvi (1902), p. 453.

⁴ 'Ibid.,' xxii (1905), p. 97.

⁵ 'American Naturalist,' xxxvi, p. 957.

⁶ 'Zool. Anzeiger,' xxvii, p. 490.

related, but in my opinion *D. laureatum* does not belong to the genus of which *D. metœcus* is the type. The widely different hosts might in the first place raise doubt as to the close relationship of the two forms, although, as Braun remarks, too great weight need not be attached to this, because the larval stages of both may be passed through in insect larvæ forming food common to bats and fishes. We may therefore neglect this.

The close resemblance between the two species, however, is not borne out on more detailed examination. Several important features of difference are apparent. The first, and possibly the chief of these, is the condition of the circum-oral swelling and papillæ. Braun did not fail to notice the difference here, but he under-estimated its importance. In *Crepidostomum metœcus* the circum-oral collar is confined almost entirely to the dorsal surface of the sucker. It does not extend on to the ventral surface, and thus its edges are at some distance from the aperture of the sucker. In *Dist. laureatum* the collar completely encircles the sucker and its edges are contiguous with, or even project into, the aperture. There are thus no ventral papillæ in *C. metœcus*, all being dorsal, or at most two being dorso-lateral. Further, they are only five in number as opposed to six in *D. laureatum*. Braun's attempt to correlate the discrepancy is certainly ingenious, and possibly explains to some extent the origin of the papillæ and the increase in their number. He considers that the median dorsal papilla in *C. metœcus* which is furcate (zweizipfelig), must be regarded as a double papilla, and as such is equivalent to the two dorsal papillæ of *D. laureatum*. The division of this median papilla would certainly make the number six, but unfortunately Braun ignores the fact that (from his own description, p. 230) the two neighbouring papillæ are not altogether simple, but appear to approach the middle papilla in shape, i. e. to be two-pointed or bi-partite. These have equal claim to be regarded as "Doppelpapillen," and in this way the number would be eight. There is no evidence of such splitting of

the papillæ in *Dist. laureatum*. On the strength of the foregoing alone I should be inclined to exclude *Dist. laureatum* from the genus *Crepidostomum*, for it is obvious that there is a wide interval of development between a condition of six and a corresponding condition of five papillæ, even although one or more of the latter is in the process of division. Further differences of generic importance are, however, not difficult to seek. The first of these is to be found in the position of the genital aperture. In Braun's figure it appears midway between the ventral sucker and the pharynx, and he describes it as close in front of the anterior border of the sucker. It is therefore considerably further back than in *Dist. laureatum*.¹ It appears single in Braun's figure. The cirrus-pouch is of great length, extending beyond the ventral sucker by nearly half the diameter of the latter. In *Dist. laureatum* it barely reaches the centre of the sucker. Braun does not offer any details of the internal structure of the pouch. A noticeable feature in *Crepidostomum metæcus* is the enormous size of the testes, each being much larger than the ventral sucker, and their approximation to the posterior end of the body. This cannot be regarded as of very great importance. Of greater moment is the position of the ovary and the condition of the uterus. In *Crepid. metæcus* the ovary lies not far in front of the anterior testis, on the right side of the body and separated from the ventral sucker by the cirrus-pouch. Braun makes no mention of amphitypy, although he had numerous specimens. The uterus is practically minimal in length and contains not more than two or three ova, which are much smaller (.055 mm.) and broader than those of *D. laureatum*. Other features of difference are the nearly equal suckers, the somewhat small, round ventral sucker, and the absence of pre-pharynx and œsophagus in *Crepidostomum*.

¹ I may be here accused of inconsistency in admitting the correctness of Braun's observation while doubting that of Olsson; but the justification lies in the fact that many errors have been found throughout Olsson's work, whereas Braun's observations are, as a rule, beyond dispute.

stomum metœcus, but the last mentioned is of little importance, for, as Braun remarked, a short œsophagus may be present as also a pre-pharynx.

The features of primary diagnostic importance are, therefore, the circum-oral collar and papillæ, the position of the genital aperture, the length of the cirrus-pouch, and the extent of the uterus with the number and size of the ova; of secondary importance are the large testes, the nearly equal suckers, and possibly the position of the ovary in *Crepid. metœcus*. That these are of much greater than merely specific importance can hardly be denied, and it follows that *Dist. laureatum* Zed., cannot be included in the genus *Crepidostomum* Braun. From the genus *Acrodactyla* Stafford¹ it differs in having the ventral sucker considerably larger than the oral sucker, the rather short cirrus-pouch, and apparently the ventral papillæ not so pronounced. The structure of the cirrus-pouch in *Acrodactyla* is not mentioned, but it is presumably muscular. From the genera *Bunodera* Raill. and *Patagium* Heymann it is sufficiently distinguished by the structure of the cirrus-pouch, the extent of the uterus, and other features to which I shall refer later. In short, it must be regarded as the type of a distinct genus, for which I propose the name *Stephanophiala*, with the following provisional definition:

Body of medium size, elongated, flattened. Cuticle unarmed. Suckers fairly muscular, the ventral sucker being larger than the oral sucker, and situated on the border of the first and second thirds of the body-length. Oral sucker subterminal, with its anterior part swollen and surrounded by a row of six equal, muscular, contractile papillæ, arising from the muscle-substance of the sucker, two being ventral and four dorsal. Intestine with short pre-pharynx, medium-sized pharynx, short œsophagus, and diverticula extending to hind end of body. Excretory vesicle simple. Genital aperture median, midway between suckers. Sinus genitalis absent or rudimentary. Cirrus-pouch with muscular walls

¹ 'Zool. Anzeiger,' xxvii, p. 491.

not extending much, if at all, beyond the ventral sucker. Vesicula seminalis simple; ductus ejaculatorius only slightly convoluted; distinct pars prostatica; prostatic cells fairly numerous. Vagina of no great length. Testes, two, rounded, close behind each other, about midway between ventral sucker and posterior end of body. Ovary rounded, not far behind ventral sucker, and separated from the testes by part of the uterus, which is of moderate length, confined between ventral sucker and anterior testis. Yolk-glands lateral, extending nearly the whole length of the body and uniting behind testes. Receptaculum seminis and Laurer's canal present. Ova not very numerous, measuring about $.075 \times .04$ mm. No intra-uterine segmentation.

Type: *St. Laureata* (Zeder). Includes also *St. transmarina* n. sp. [= *Crepidostomum laureatum* (Zed.) Stafford, 1904], and probably two other undescribed American forms (see p. 35).

With regard to the systematic position of the genus it probably lies nearer *Acrodactyla* than *Crepidostomum*. From *Bunodera*, *Patagium*, *Rhytidodes* and others it is much further removed. Heymann has already discussed the relationship of the genus *Crepidostomum* to *Bunodera* and *Patagium*. The somewhat scanty knowledge of the first-named genus did not appear to offer any serious obstacle to the inclusion of these three genera under the subfamily BUNODERINÆ, and, in addition, the importance of the recognised features of difference was under-estimated, as will be evident from what follows.

The presence of circum-oral swellings or papillæ being a rare and peculiar occurrence amongst Distomids, the most natural conclusion is that they must have been evolved from some simpler type. That they bear no analogy to the much commoner collar of cephalic spines of the ECHINOSTOMINÆ and other forms has already been amply demonstrated by Looss, and needs no further discussion here. The resemblance, however, in the internal anatomy of the genera under consideration, especially of *Stephanophiala*, to the ALLO-

CREADIINÆ and allied forms has previously been remarked on by more than one author, and there is certainly no other known group to which they seem so closely connected. *Bunódera* and *Patagium*, nevertheless, diverge widely from the ALLOCREADIINÆ in several respects. On the other hand, were it not for the circum-oral papillæ, *Stephanophiala laureata* might be regarded as an Allocread.

From the point of view of the circum-oral collar, the genera *Patagium* and *Rhytidodes* Lss. show the most primitive condition of a simple swelling of the muscle-substance of the sucker with commencing differentiation. The next stage, considerably removed, is that of *Crepidostomum* in which papillæ have appeared, showing a tendency to further subdivision, and the most advanced stage is that of *Stephanophiala*, *Bunodera* and *Acrodactyla*, in each of which the condition is very similar, although, as I have tried to show, they may not be the direct evolutionary products of forms such as *Crepidostomum*, but may have proceeded along independent lines, the intermediate members of which have been lost or are not yet known. A consideration of the internal anatomy leaves hardly a doubt of this, for *Stephanophiala* and *Bunodera* differ very much, while the former approaches *Crepidostomum* and the latter *Patagium*.

From a detailed examination of the internal structures it is evident that *Stephanophiala* closely resembles the ALLOCREADIINÆ in its most essential features, viz. the position of the genital aperture, the structure of the cirrus-pouch and its contents, the position of the testes, the extent and situation of the uterus, and the condition, size and number of the ova. This appears to have been noticed by Pratt¹ who included *Crepidostomum* Brn. in a sub-family, PSILOSTOMINÆ Pratt, comprising *Allocreadium* and several allied genera,² while

¹ 'American Naturalist,' xxxvi, p. 888.

² Pratt is rather inconsistent, for on p. 896 he notes: "Yolk-glands do not extend in front of acetabulum" as a feature of the sub-family PSILOSTOMINÆ, while just above he has—"Yolk-glands extend in front of acetabulum," under which sub-division he puts *Calycodes* Les.,

Bunodera is relegated to a separate sub-family, *BUNODERINÆ* Pratt, including the rather anomalous genus *Tergestia* Stoss.

In *Crepidostomum* the uterus appears to have undergone a process of retrogression or degeneration, while *Bunodera*, and, to a less degree, *Patagium* display a condition altogether foreign to the *ALLOCREADINÆ*, in which the uterus never extends beyond the anterior border of the first testis. In *Crepidostomum* and *Acrodactyla* the structure of the cirrus-pouch is not well known, but indications point to its being not unlike that of *Stephanophiala*. *Bunodera* and *Patagium* again display a distinctly different type, with non-muscular wall and more or less highly-convoluted vesicula seminalis. The absence of muscle-fibres might be regarded as the result of a series of degenerative changes, but that is merely hypothetical. Further, in *Bunodera* the condition of the ova, in which a *Miracidium* larva is developed within the uterus, is very dissimilar from that of the *ALLOCREADINÆ* and *Stephanophiala*. In *Crepidostomum* and *Patagium* the condition of the ova is not known.

The inclusion, therefore, of all these genera within the sub-family, *BUNODERINÆ*, as proposed by Looss and Heymann, is not without objection. They are possibly related, but how closely is not very apparent. Too great weight has hitherto been placed on their common possession of a circum-oral collar, and, as in the case of *Rhytidodes*, such a structure may be present in a genus which cannot be included in the same sub-family. To consider the circum-oral collar as the diagnostic feature of the sub-family *BUNODERINÆ* would be a reversion to the now discarded system of classification by external characters. We must therefore look to the internal structure, mainly, for guidance. From this it is evident

Crepidostomum Brn. and *Helicometra* Odhn., although he includes those genera in his sub-family. Pratt's classification is obviously premature but it is of great help, and he is certainly correct in separating *Bunodera* from the *PSILOSTOMINÆ*.

that a somewhat broad line of separation exists between *Stephanophiala*, *Acrodactyla* and *Crepidostomum* on the one hand and *Bunodera* and *Patagium* on the other. It is unfortunate that more details are not available in the case of *Crepidostomum*, *Acrodactyla*, and *Patagium*, but from what is actually known it is highly probable that they display all the features mentioned in the following table :

	<i>Stephanophiala</i> (<i>Crepidostomum</i> and <i>Acrodactyla</i> ?).	<i>Bunodera</i> (and <i>Patagium</i>).
1. Testes	. Directly tandem	. Oblique
2. Uterus	. Confined between ventral sucker and anterior testis	. Extending beyond testes, and even filling up posterior part of body.
3. Cirrus-pouch	. Muscular; vesicula seminalis and ductus only slightly convoluted	. Membranous (non-muscular); vesicula and ductus highly convoluted.
4. Ova	. No intra-uterine segmentation.	<i>Miracidium</i> larva developed within uterus.

The difficulty again arises as to the precise importance to be attached to these differences in structure. From a study of the methods of classification propounded and developed by Looss I take it that the condition of the ova and the cirrus-pouch are of extreme importance, and that genera differing in two such essential features can hardly be included in the same sub-family. On examination of already existing and strictly demarcated sub-families it will be found that no such divergence in structure is to be met with. Amongst such sub-families, for example, as the *ECHINOSTOMINÆ*, *ALLOCREADINÆ* or *GORGODERINÆ* with their comparatively numerous members no such difference in structure would be admitted. From these considerations, therefore, it seems advisable to separate the genera *Stephanophiala* and *Crepidostomum* from the sub-family *BUNODERINÆ*,

and to establish for them a distinct sub-family for which I propose the name *STEPHANOPHIALINÆ* n. subfam., with the following provisional diagnosis.

Small to under middle-sized forms with fairly muscular body, the anterior part of which is capable of considerable extension. Cuticle without spines or scales. Suckers muscular and of moderate size, the ventral sucker being situated at the end of the anterior third of the body or somewhat further back. Oral sucker with a circum-oral collar developed from the muscle substance of the sucker, from which a number (five or six) of small tentacular papillæ arise. Intestine with very short pre-pharynx, muscular pharynx, short œsophagus, and long simple diverticula extending to posterior end of body. Genital aperture median, between the suckers. Sinus genitalis small or vestigial. Cirrus-pouch elongated, with muscular walls. Vesicula seminalis and ductus ejaculatorius simple and not much convoluted. Testes two, simple, median in the middle of the post-acetabular region. Ovary simple, rounded, not far behind ventral sucker and separated from testis by part of uterus. Receptaculum seminis and Laurer's canal present. Yolk-glands mainly lateral, extensive. Uterus confined between anterior testis and ventral sucker. Vagina simple, short. Ova not numerous, measuring about .05-.08 mm. No intra-uterine segmentation.

Type: *Stephanophiala* Mihi. Including also probably *Crepidostomum* Brn., and very doubtfully *Acrodactyla* Stafford.

As a result of this separation Heymann's definition of the sub-family *BUNODERINÆ* Lss. requires modification. The sub-family must for the present be restricted to the genera *Bunodera* and *Patagium*, and it is obvious that these genera differ from each other much more than *Crepidostomum* and *Acrodactyla* do from *Stephanophiala*, not only as regards the circum-oral collar but also in respect of the internal structure. The modifications, as already indicated, are: Suckers nearly equal; testes oblique; uterus extending

beyond the testes; terminal part of male genital apparatus enclosed within a non-muscular pouch; vesicula seminalis convoluted; ovary immediately behind ventral sucker and separated from testes by considerable part of uterus; ova contain a *Miracidium* larva before deposition. Length .08-.10 mm.

Type: *Bunodera* Raill.

In addition to the species hitherto mentioned there are several other forms, mostly from America, which possess a row of circum-oral papillæ. Two of those, *Distomum auriculatum* Wedl. and *D. petalosum* Lander, were regarded by Looss¹ as probable members of the genus *Bunodera* Stafford,² however, differentiated *D. petalosum* Lander (= *D. auriculatum* Wedl. Linton) from *Bunodera*, and proposed it as type of the new genus *Acrodactyla*. The chief features of this genus, according to Stafford, are the large ventral oral papillæ, the position of the genital aperture, and the large cirrus-pouch. To these I should add the fact that the ventral sucker is smaller than the oral. The structure of the cirrus-pouch and the condition of the ova are not noted. The limited extent of the uterus indicates that this genus is more nearly related to *Stephanophiala* and *Crepidostomum* than to *Bunodera*, and I have included it provisionally within the sub-family STEPHANOPHIALINÆ.

With regard to the American forms of *Distomum laureatum* described by Linton and Stafford, it is doubtful if they are really identical with the European variety. The only differences which I can detect in Linton's specimens are the backward position of the ovary and the slightly reduced number of ova. In Olsson's figure of the species, however, the ovary is a little distance behind the ventral sucker, so that this variation may occur in the European form. The smaller number of ova may be only a seasonal variation. Linton makes no mention of the cirrus-pouch, but Stafford says it

¹ 'Zool. Jahrb. Syst.,' xvi, p. 453.

² 'Zool. Anzeiger' (1904), xxvi, p. 491.

extends beyond the ventral sucker. The latter also describes the ovary as midway between ventral sucker and anterior testis, and the ova as not numerous—tento twenty. In this case it is apparent that a different species is under consideration, for in the European form, according to Olsson's observation, which I confirm, the cirrus-pouch does not extend beyond the centre of the ventral sucker. For this American species I propose the name *Stephanophiala transmarina* (= *Crepidostomum laureatum* Stafford, 1904, = ? *Dis-tomum laureatum* Linton, 1893) from *Salvelinus fontinalis* Mit. and *Salmo mykiss*.

Stafford mentions two other varieties, one from *Perea flavescens*, the other from *Necturus maculatus*, and these will possibly prove to be further species of the genus *Stephanophiala*. With regard to *Crepidostomum cornutum* (Osborn),¹ it is doubtful if this species can be included in the genus *Stephanophiala*. The small size of the ventral sucker is against this and brings it into closer relation with *Acrodactyla*. The species, however, is not yet sufficiently well known for its position to be determined. If it cannot be included in the genus *Acrodactyla* it may require to be considered as the type of a distinct genus.

The natural sequel to the foregoing classification would be the formation of a family BUNODERIDÆ along the lines indicated by Heymann in his definition of the sub-family BUNODERINÆ, but such a step would be undoubtedly premature, if not erroneous. It must be regarded as very doubtful if the STEPHANOPHIALINÆ are so closely related to the BUNODERINÆ, as has been hitherto considered. Most indications seem to point to a further separation of these two sub-families, and a nearer approximation of the STEPHANOPHIALINÆ to the ALLOCREADINÆ. This, however, must be a matter for future determination.

¹ 'Science,' xv (1902), p. 573.

Sub-family BRACHYCLADIINÆ Odh., 1904.

Genus *Brachycladium* Lss., 1899.

Brachycladium oblongum (Braun). Pl. 9, figs. 6—9.

? = *Campula oblonga* Cobbold.

1900. *Campula oblonga* Cobb. Braun, 'Centralbl. f. Bakter.,' xxviii; 'Abtheil.,' i, pp. 249–255, 3 figs.

1902. *Brachycladium oblongum* (Brn.), Looss, 'Zool. Jahrb. Abth. Syst.,' xvi, pp. 707–717 and 775–778.

1904. *Brachycladium oblongum* Odhner, 'Die Trematoden des arktischen Gebietes, Fauna Arctica,' iv, p. 347.

This species has been the cause of much systematic discussion, and its ultimate fate may be regarded as a critical test of the priority law as applied to zoological nomenclature. The root of the trouble lies in the fact that Cobbold's type specimens of *Campula oblonga* have been lost or destroyed, and that his description¹ of the species is inadequate to differentiate it from allied and more recently discovered species. I have made an exhaustive but fruitless search for Cobbold's specimens, and it may certainly be considered that they no longer exist, unless, perchance, in some private collection. What remains to us, therefore, of Cobbold's species is merely his scanty description, which, as Looss justly remarks, is of no value whatsoever for diagnostic purposes. Braun's identification, as *Campula oblonga* Cobb., of a species which he found in the same situation in the same host as Cobbold found his specimens, is regarded by Looss as inadmissible, and his opinion is seconded by Odhner. Braun defended his diagnosis on the ground of the similarity of habitat and the manifest resemblance between his specimens and Cobbold's description and figure so far as they went, but Looss pointed out that *Brachycladium palliatum* Lss., *Br. rochebruni* Poir., and *Br. delphini* Poir. resemble Cobbold's *Campula oblonga* just as closely as do Braun's specimens, and so might just as readily be

¹ 'Trans. Linn. Soc. Lond.,' xxii (1858), p. 168, Pl. XXXIII, figs. 84, 85.

regarded as *Campula oblonga*. The obvious outcome of this is that *Campula oblonga* Cobbold must be considered a nomen nudum, and that Braun's specimens must be re-named *Brachycladium oblongum* (Braun). I am able to offer a justification, in part, of Looss's conclusions, for in a female porpoise which I have recently (August, 1908) examined I found in the bile-ducts, in addition to numerous examples of *Brachycladium oblongum* (Brn.), a single specimen of another Trematode. This was accidentally discovered on washing the parasites, and, in spite of further repeated search no more specimens could be obtained. It was easily differentiated from *Brachycladium oblongum* by its deep red colour, more elongated body, and the apparent absence of spines. I have not yet identified this specimen, but it appears to be a species of *Brachycladium*. This species could not possibly have been the one Cobbold found, but its occurrence shows that more than one Trematode species may have as its habitat the bile-ducts of *Phocæna communis*.

The first specimens which I had the opportunity of examining formed part of Professor McIntosh's collection, and were obtained by him from the liver of a porpoise shot in Lochmaddy (North-West of Scotland) in April, 1865. About fifty examples were found, and they were identified by Professor McIntosh at that time as *Campula oblonga* Cobbold. As already mentioned I have obtained the same parasite myself from one of two porpoises captured in the Firth of Clyde, near Millport. The first of these, a young male, was unaffected, but in the second, one of the lobes of the liver contained more than a hundred specimens. The presence of the parasites in the liver was indicated by large yellowish prominences on its surface, which felt extremely hard. The same stony hardness could be felt throughout the substance of the affected lobe. On opening into it the biliary canals were found lined with a thick layer of dense fibrous tissue. The terminal parts of the canals were usually dilated to form small sacs, in which large numbers of the parasite were closely packed together. These were, however, easily pressed out. Detailed examination has

shown these specimens to be identical with the species described by Braun, or, at most, to differ very slightly from it. Braun's description deals only with the more obvious anatomical features, and I propose here to describe the structure in fuller detail. Looss has already¹ given a fairly exhaustive account of the anatomy of *Brachycladium palliatum*, and it may be of interest to see in how far his results are borne out by my observations.

The specimens obtained from Lochmaddy were well preserved in alcohol and most of them were in a moderate state of extension, although few were so greatly extended as the specimen shown in Braun's fig. 3. The most general length was 5-6 mm., and the breadth of the specimens was 2-2.5 mm., i. e. the length is about two and a half times the breadth. My own specimens are more extended. Many of them reached 7 mm. in length, and the length was three to three and a half times the breadth. This agrees with Braun's observations.

The other *Brachycladium* spp. are much more elongated than this, the length being in the case of *Br. rochebruni* ten times the breadth, while in *Br. palliatum* it is five or six times. The greatest thickness in my specimens was .75-.85 mm. The outline is broadly lanceolate, being somewhat pointed at each end. The greatest breadth occurs just behind the ventral sucker, the greatest thickness at the level of the genital aperture.

The whole body is covered with a thick cuticle, varying from .008-.013 mm. in thickness, but not in any uniform manner, thicker and thinner parts alternating with each other, and there being no difference in this respect between ventral and dorsal surfaces. The cuticle is much thinner than that of *Br. palliatum*. Strong spines stud the entire surface of the body, being present right to the posterior end, and even in a small depression on which the excretory vesicle opens. As usual they vary much in size, the largest spines being found about the thickest part of the body and the size gradu-

¹ 'Beiträge z. Kenntniss der Trematoden. Zeitschr. f. wissen. Zoolog.,' xli (1885), pp. 390-427, figs. 1-14 and 30.

ally diminishing forwards and backwards. Around the oral sucker they are $\cdot 019$ mm. long with a base measurement of $\cdot 0045$ mm. The maximum size about the middle of the body is $\cdot 058$ by $\cdot 014$ mm. Towards the posterior end they measure $\cdot 037$ by $\cdot 012$ mm. They are thus much shorter than the spines of *Br. palliatum*. The spines are deeply embedded in the cuticle, penetrating its whole thickness and causing bulging of the basement membrane. Immediately beneath the latter lie the usual three layers of muscle-fibres. The circular fibres have a diameter of $\cdot 0019$ mm., and are separated by spaces equal to their diameter. The longitudinal fibres measure $\cdot 003$ – $\cdot 004$ mm., and are separated by spaces of $\cdot 006$ – $\cdot 01$ mm. The diagonal fibres measure $\cdot 0035$ – $\cdot 0055$ mm., and are widely separated by spaces of $\cdot 04$ mm. Those passing in opposite directions make an angle of 125° with each other, but this varies with the state of contraction. The longitudinal and diagonal fibres run for the most part in pairs, which are in more or less intimate contact. Sometimes they are indistinguishable; sometimes a distinct interval separates them. This corresponds with the condition described by Looss in *Br. palliatum*. In both species the circular fibres run singly.

The suckers are small in comparison with the size of the animal. Both are globular with circular apertures, and present no peculiar feature. The oral sucker is subterminal and has a diameter of $\cdot 33$ mm. The ventral sucker is situated at a distance of rather more than a quarter of the body length from the anterior end, and has a diameter of $\cdot 43$ – $\cdot 46$ mm. The diameters are therefore in the proportion of 3:4, and are respectively about one sixteenth and one twelfth of the body length (taking the average as $5\cdot 5$ mm.). This agrees fairly well with Braun's measurements.

The radial fibres of the oral sucker are $\cdot 015$ mm. thick; those of the ventral sucker $\cdot 025$ mm., the circular fibres about $\cdot 019$ mm. The wall of the oral sucker has a thickness of $\cdot 078$ mm., that of the ventral sucker $\cdot 09$ mm. The cuticular lining of the suckers is thinner than the cuticle covering the body, being only about $\cdot 004$ mm. thick.

The mouth opens into a short but wide pre-pharynx. From the ventral side of this there arises a well-marked pre-pharyngeal diverticulum (fig. 8), which passes backwards ventral to the pharynx, and may extend a short distance beyond it. This diverticulum forms part of the pre-pharynx and there is no difference in its structure; its occurrence, however, is constant. Such a structure appears to be absent in *Br. palliatum*, and was not observed by Braun or Poirier. Its function is not very apparent, but it is a curious fact that in each of three specimens which I examined a number of ova were found in the diverticulum. Ova, however, were found in one case in the intestinal diverticula, and were probably accidentally swallowed. The width of this pre-pharyngeal pouch is $\cdot 13$ – $\cdot 18$ mm., but at its junction with the pre-pharynx it is only $\cdot 09$ mm. Its walls are in some cases thrown into irregular folds, but they are not muscular.

The pharynx is somewhat flask-shaped, narrowest in front and with its anterior end projecting far into the pre-pharynx. The extreme freedom of movement of the pharynx is evident from the fact that its anterior end may be found directed ventrally, dorsally or towards either side. Its length in a $5\cdot 5$ mm. specimen is $\cdot 36$ mm.; breadth $\cdot 22$ mm. and thickness $\cdot 20$ mm. It is, therefore, a comparatively large structure, and is narrower than that of *Br. palliatum* and larger in proportion to the size of the animal. The histological structure is the same as that of the suckers. The myoblasts measure $\cdot 008$ – $\cdot 011$ mm., with minute reddish eccentric nucleoli.

The œsophagus arises from the posterior end of the pharynx as a narrow tube, transversely oval in section, with a diameter of $\cdot 10$ mm. It gradually increases in diameter to $\cdot 13$ mm., where, at a distance of $\cdot 05$ mm. behind the pharynx, there is a small dilatation on the left which causes the width to increase to $\cdot 20$ mm. The bifucation takes place at a distance of $\cdot 115$ mm. behind the pharynx. This, however, is not a bifurcation in the usual sense of the term, when the

diverticula separate from each other at a somewhat wide angle; it is rather a division of the œsophagus into two by a thin septum. The two parts run side by side and their lumen is still lined with "cuticle." Intestinal epithelium does not appear till a further distance of .05 mm., and immediately thereafter the diverticula proper arise. This structural peculiarity of the œsophagus has not been noted by any previous author and is not indicated in Braun's figures. It is probable, however, that the small transverse parts in *Br. palliatum* and the other two species, joining the undivided œsophagus to the main diverticula correspond with the parts which I have described above, and that they are lined by cuticle and therefore functionally part of the œsophagus. Around the anterior end of the œsophagus are several groups of rather large cells, which probably correspond to the salivary glands of *Br. palliatum* as described by Looss.

The diverticula occupy the lateral fields midway between dorsal and ventral surfaces and extend to within a short distance of the posterior end of the body, turning in towards the middle line at their termination. In addition to the main diverticula a pair of smaller diverticula, one on each side, pass forwards and reach almost the level of the middle of the oral sucker. These secondary diverticula are distinctive of the genus. The condition of the diverticula does not differ from that in the other species. They pursue a very erratic course, and numerous dilatations of various sizes, never attaining the size of twigs, are found both on their outer and inner sides. The sinuities are so numerous and irregular that on section the diverticula present the appearance of being traversed by trabeculæ. The condition suggests that the diverticula, originally simple, had grown too long for the body and had become at first regularly sinuate and then crushed up to be accommodated in the limited space.

The structure of the intestinal wall is identical with that described by Looss. The epithelium is well developed and presents numerous villus-like projections. The muscular

coats of the œsophagus are well marked but those of the diverticula are thin.

As already noted the excretory aperture opens on a large pit-like depression at the posterior end of the body; whether this depression is an intrinsic part of the excretory system or not I am at a loss to say. Small cuticular spines penetrate a short distance within it, and the rest of its surface is lined by a thin membrane which appears like cuticle but is much thinner than the body cuticle. It may be either an invagination of the posterior end of the body, or a dilatation of the terminal part of the excretory duct, or both combined. A similar condition has not been observed in the other species of the genus, and as it is only visible on section Braun was not able to observe it. It was present in each of my specimens. Its significance is not apparent.

A narrow tube with muscular walls leads from the aperture to the vesicle. Several muscle-fibres are grouped round the aperture in the form of a sphincter. The vesicle itself is of simple structure. It is lined throughout by a thin, delicate membrane, in which small scattered epithelial cells can be observed. The wall is extremely irregular, being thrown into innumerable little wrinkles and folds. There is a deposition of muscle-fibres, both annular and longitudinal, but they are very fine. The vesicle lies in a mass of very loose connective tissue, and it extends as far forward as the posterior border of the ovary, passing dorsally to the testes, by which it is compressed, but expanding in the spaces between the testes and ovary. The anterior end is flattened and broadened and into it the two lateral tubules enter. These pass forwards and are distributed in the manner which Looss has fully described in *Br. palliatum*.¹

The genital aperture is median, close in front of the ventral sucker. It is a narrow transverse slit with sphincter and radiating muscle-fibres. The genital sinus is of small size. The male duct opens into it on the right, the female on the left. The radial fibres of the genital aperture pass into the

¹ Op. cit., p. 405

longitudinal fibres of the cirrus-pouch and vagina. The cirrus-pouch (fig. 7) is a pear-shaped or ovoid structure extending backwards nearly as far as the posterior border of the ventral sucker. It thus reaches further back than in any other species of the genus. At its posterior end it approaches the dorsal surface of the body, and is separated from the ventral sucker by the uterus. Its maximum diameter is .3 mm. The longitudinal muscle-fibres of its wall have a diameter of .002–.004 mm., and are separated by spaces of .001–.004 mm. The annular fibres are much finer, .0004–.002 mm., with intervening spaces of .0007–.0015 mm. The vesicula seminalis is of large size and occupies the greater part of the cirrus-pouch. Its length is about .8 mm., but it displays numerous dilatations and convolutions. Its diameter varies from .16 mm. to .28 mm. At its anterior end its wall is slightly invaginated into the pars prostatica, which is a short S-shaped tube with a diameter of .066 mm. The prostatic cells are comparatively few and are entirely confined to the space around the pars prostatica. The cells are oval, with a long diameter of .012 mm. and nuclei of .0035–.004 mm. diameter. Amongst the prostatic cells there are a few larger cells measuring .019 mm., which do not stain so deeply and have faintly-staining, round nuclei measuring .0062 mm. At its anterior end the pars prostatica passes into a widely dilated ductus ejaculatorius, which presents a rather unusual condition. In its retracted state, instead of being convoluted or wound upon itself, as is the case in many species, it is crumpled up (concertina-fashion). In sections it presents a grating-like appearance (fig. 7), the lumen being alternately contracted and expanded. The crumpling, however, is not by any means irregular, fairly equal spaces being maintained between the folds.¹ The structure of the wall of the ductus does not differ from that commonly met with, there being an

¹ A somewhat similar condition appears to exist in *Distomum alacre* Lss., according to Looss's short description of that species ('Centralbl. f. Bakter.,' Abth. 1, vol. xxix, p. 401). In the genera *Fellodistomum* and *Steringophorus* an analogous condition exists, but in these the ductus is very short.

inner layer of well-marked annular muscle-fibres and an outer layer of less distinct longitudinal fibres. The lumen is lined by a rather thick cuticularised epithelium. In addition, however, to the two intrinsic muscle layers there are numerous other stout muscle-fibres passing from the ductus to the wall of the cirrus-pouch. These run more or less obliquely, but on eversion of the ductus they are drawn nearly parallel to it. To explain this peculiar condition of the ductus it seems necessary to consider that these extrinsic muscle-fibres exert the most important action in retracting the ductus from its everted position and that on account of their direction they cause it to fold up instead of winding on itself. What action the intrinsic longitudinal fibres take or why the eventual result should be so different from that most frequently observed are questions not easy to determine. No mention is made of such a condition by Looss in the case *Br. palliatum*. His figure (Pl. XXIII, fig. 8) represents the ductus as a slightly tortuous tube of uniform calibre, widening somewhat suddenly as it passes backwards and surrounded by a closely packed mass of gland-cells (Anhangsdrüsen). The condition is therefore totally different from that in *Br. oblongum*, and it is a curious fact that two so closely related species should present such an important feature of difference. Cells which stain deeply (gland-cells or myoblasts) are certainly present around the ductus, but they are comparatively few in number and scattered in the midst of looser tissue.

That the terminal part of the ductus functions as an exsertile cirrus there can be no doubt, for in one of my specimens I found it extended about .06 mm. beyond the genital aperture (i. e. about .2 mm. from the male aperture). In that case the anterior folds of the ductus were straightened out, but the posterior folds remained, so that while the anterior part became a tube of uniform calibre, the posterior part remained in the condition above described.

The histological structure of the pars prostatica and vesicula seminalis does not differ from that in *Br. palliatum*.

The lining epithelium appears to be of somewhat lower type, and the muscle-layers are rather finer.

From the posterior end of the vesicula seminalis a single vas deferens, .023 mm. in diameter, passes backwards to the level of the middle of the ovary, where it divides into the paired vasa. These remain close together for a short distance and then separate to enclose the ootype. They join the testes on their dorsal surface.

The testes are median, close together, and directly behind each other. They occupy almost completely the middle third of the body, the posterior testis being exactly one third from the posterior end of the body, and the anterior testis a slightly greater distance from the anterior end. Each has a rather peculiar shape, differing from that of the other. The anterior testis is twice as broad as it is long, and its thickness equals its length. It is therefore much compressed in the long axis of the body; not only so, but there is a distinct hollowing out both on the anterior and posterior surfaces, which is obvious only on longitudinal section. It consists of four lobes, two large lateral with small anterior and posterior lobes. The posterior testis is much more symmetrical, being composed of four nearly equal lobes—an anterior, a posterior, and two lateral. The anterior lobe is slightly indented on its ventral aspect, and the posterior shows one or two irregularities, but the lateral lobes are almost uniformly rounded. The lobing is quite deep, and the central part of the testis is about equal in size to each of the lobes. This testis is almost iso-diametric, and its maximum thickness is about half its diameter. The thickness diminishes from the centre to the periphery. This symmetrical shape might be regarded as the normal condition of the testes, and the shape of the anterior testis as the result of deformity. In Braun's figure (fig. 3) the testes are shown as irregularly four-lobed bodies. The particularly long posterior lobe of the hinder testis, to which Braun draws attention, is evidently not a constant feature. In *Br. palliatum* the testes are lobed, but the lobes are smaller, more numerous, and do not appear

to have any constant direction. In *Br. delphini* and *Br. rochebruni* they are regularly ovoid. In a specimen of 5.5 mm. length the sizes of the testes are: Anterior, length .54 mm., breadth 1.20 mm., thickness .55 mm.; posterior, length .93 mm., breadth 1.02 mm., thickness .52 mm. Thus the diameter of the posterior testis is two elevenths of the body length.

The ovary is median or very slightly to the right side, directly in front of the anterior testis and close to the ventral surface of the body. It is separated from the ventral sucker by a very short space. Braun is not quite correct in saying that the ovary is globular in shape, for I find it constantly slightly elongated transversely. The mean of three measurements gives the length .30 mm., breadth .37 mm., thickness .28 mm. The ovarian ova are largest towards the anterior-dorsal surface, and measure $.0155 \times .0087$ mm. They are fusiform in shape, with large, round nuclei, measuring .0077 mm. Looss has figured and described the corresponding cells of *Br. palliatum*, and it is evident that our observations agree fairly closely, except that I find the cells constantly rather more elongated. It is true, however, as Looss remarks, that these cells undergo various changes in shape.

The shell-gland complex (fig. 6) in this species differs in more than one respect from that of *Br. palliatum*, as described by Looss, and particularly in the greatly reduced size of the receptaculum seminis and the presence of a distinct yolk-reservoir. The oviduct (diameter .029 mm.) arises from the dorsal surface of the ovary. Not far from its origin it describes a complete circle upon itself and then passes on in a straight course. About midway between the ovary and the dorsal surface of the body it dilates slightly and then turns at right angles towards the left. At this point there is a small sacculation, on the right side, no wider than the oviduct itself, and not differentiated from it by any marked constriction. This is all that remains of the receptaculum seminis, and in all probability it no longer functions as such, for it never contains sperms and its lumen is almost entirely

filled up with the hair-like processes of the epithelial cells. Moreover, its function seems to have been undertaken by the initial part of the uterus, which in every specimen is packed full of sperms (receptaculum seminis uterinum). Almost immediately beyond the above-described turning of the oviduct Laurer's canal arises. It has a peculiar and almost constant course. It passes first towards the dorsal surface, bending slightly to the left, but after traversing half the distance it turns suddenly at right angles and runs posteriorly parallel to the dorsal surface for a considerable distance, describing at the same time almost a complete semi-circle with its convexity towards the right. It then again turns abruptly at a right angle and passes in a more or less direct course towards the dorsal surface, where it opens almost in the middle line on the level of the anterior border of the anterior testis or a little further forwards. Its diameter is about .023 mm.

From the origin of Laurer's canal the oviduct proceeds on its way towards the ootype, running almost parallel to the surface of the ovary. Just before entering the ootype it receives the common yolk-duct, which passes forwards in a rather sinuous course from a small reservoir lying a little in front of the anterior testis and almost midway between the ovary and the dorsal surface. The ootype, which is situated dorsal to the left end of the ovary, bends forwards and dorsally to pass into the uterus. The ootype is wider (diameter .033 mm.) than the oviduct and the uterus is still wider. The shell-gland is of large size. It does not invest the ootype closely, but its cells are diffusely scattered in the surrounding parts and communicate with the ootype by means of long ducts. They are most numerous around the oviduct and common yolk-duct. The histological structure of the shell-gland complex conforms to the usual type. The whole course of the oviduct is lined by a thick ciliated epithelium. The cilia are fairly long and are directed away from the ovary. The receptaculum seminis is also lined by this ciliated epithelium, the cilia of which are so numerous that they

almost obliterate the cavity of the receptaculum. At irregular intervals in the epithelium of the oviduct there are single large cells, evidently the same as those observed by Looss in *Br. palliatum*.¹ For a very short distance Laurer's canal is also lined by ciliated epithelium, the cilia of which are directed towards the ovary, i. e. in a direction opposite to that of the cilia in the oviduct. It is evident that this arrangement has some relation to the prevention of the escape of ova or sperms through Laurer's canal. An analogous condition has been observed by Looss in *Distomum unicum* Lss.² The remainder of Laurer's canal is lined by a cuticularised epithelium. The ootype is lined by a regular non-ciliated epithelium. The cells of the shell-gland do not have the dense arrangement represented by Looss in *Br. palliatum*.³ They are fairly large cells, measuring $\cdot 020$ by $\cdot 012$ mm., and nuclei $\cdot 005$ mm.

The yolk-glands are richly developed, extending from the level of the pharynx to the posterior end of the body. They are mainly lateral, but their situation is not so much marginal as ventral and dorsal to the intestinal diverticula. Behind the testis they unite in the middle line, also to a slight extent in front of the genital aperture. The follicles are not very large, about $\cdot 07$ – $\cdot 08$ mm. diameter. The component cells are not well differentiated, owing to their containing a large number of bright yellow refracting granules, which are about $\cdot 003$ mm. in size. These granules are very difficult to stain, remaining untouched long after the other tissues are deeply stained, but eventually they do take on the stain. I found that they did so more readily with saffranin and toluidine-blue than with hæmalum and eosin. The small ($\cdot 004$ mm.) round nuclei of these cells stain readily enough. I was quite unable to determine any differentiation between central cell and peripheral cells as in *Stephanophiala*

¹ Op. cit., pl. xxiii, fig. 10.

² "Recherches sur la faune parasitaire de l'Egypte"; in 'Mém. Inst. Egypt,' iii (1896), p. 49.

³ 'Zeitsch. f. wissen Zool.,' xli, pl. xxiii, fig. 13.

laureata. The transverse ducts run along the anterior border of the anterior testis to unite in the small median reservoir. This contains a small number of yolk-cells in which the nuclei are still present. The common yolk-duct passes forward to join the oviduct as already described.

It appears to be quite erroneous to speak of the secretion of the yolk-glands, as so many observers have done, for as I have shown both in the case of *Stephanophiala laureata* and the present species, the yolk substance is the result, not of a process of secretion, but of a process of proliferation of formative cells in a manner somewhat analogous to the production of ovarian ova or spermatozoa. The yolk-glands must therefore not be regarded in the same category as the shell-gland and prostate-gland, which produce a true secretion. They are glands only in the same sense as the ovary and testes are glands.

As in *Br. palliatum*, the uterus is confined to a very limited space between the ovary and the level of the genital aperture and between the intestinal diverticula on either side. It thus lies dorsal to the ventral sucker and occupies the entire thickness of the body. Its course is impossible to determine, owing to the greatly convoluted condition. The initial part, from the ootype, is lined with a highly ciliated membrane, but the cilia disappear after a short distance. The uterus then becomes distended with ova, which are surrounded by a countless number of sperms. This condition persists for about a third of the total length of the uterus, and it is evident that we have here to deal with a true receptaculum seminis uterinum, which is met with in several other species and which performs the function of the vestigial receptaculum seminis proper. The condition here is evidently much removed from that in *Br. palliatum*, in which, according to Looss, the receptaculum seminis is full of sperms, and performing its true function, while no mention is made of a receptaculum seminis uterinum.

The uterus terminates near the dorsal surface of the body, and the vagina passes almost in a straight line, dorso-

ventrally, towards the genital sinus. It has a length of .45 mm., and a diameter of .04-.09 mm., becoming narrow as it approaches the genital sinus. Its section is not circular, but triangular, and this is constant in every case, being apparently in correlation with the triangular shape of the ova. Its wall is thick (.012 mm.) and muscular, comprising the usual two layers, and it is lined by a thick cuticular membrane.

The ova are fairly numerous and measure .084 by .0436 mm. The shell is light yellow and about .0035 mm. thick. The shape is characteristic. In longitudinal section they are somewhat oval, but truncate at the anterior end and with a pointed knob, due to a thickening of the shell, at the posterior end. The operculum is very flat. In transverse section, however, they are triangular, the triangle being equilateral. This peculiar shape of the ova was first noted, I believe, by Odhner¹ in the case of *Orthosplanchnus arcticus* Odh., *O. fraterculus* Odh., *Lecithodesmus goliath* (v. Ben.), and *Brachycladium oblongum* (Brn.). In his diagnosis² of the sub-family BRACHYCLADIINÆ Odh. he regards it as a distinctive feature differentiating the sub-family, amongst other things, from the FASCIOLINÆ. He thus considers it to be of universal occurrence in the sub-family, and in particular in the genus *Brachycladium* Lss. He refers³ to Poirier's⁴ figures of the ova of *Br. rochebruni* and *Br. delphini* as indicating that the ova of the species probably display the same shape. I fail to see any evidence of this in Poirier's figures, and he himself certainly makes no reference to the triangular shape of the ova in transverse section.⁵ Odhner conveniently ignores Looss's statement⁶ that

¹ "Die Trematoden des Arktischen Gebietes" in 'Fauna Arctica,' iv, p. 343.

² Ibid., p. 347.

³ Ibid., p. 346.

⁴ "Trematodes nouveaux ou peu connus," in 'Bull. Soc. Philomatique, Paris,' sér. 7, vol. x, pl. iv, figs. 3 and 5.

⁵ It is quite possible that Odhner only means to imply that the ova in Poirier's two species resemble those of the other BRACHYCLADIINÆ in being thick shelled, with flat operculum and pointed posterior end, but his statement in the sub-family diagnosis renders this doubtful.

⁶ 'Zeitsch. f. Wissen. Zool.,' xli, p. 419.

the ova in *Br. palliatum* "haben fast die Gestalt eines Rotationsellipsoides." His hypothesis, therefore, although highly probable, requires confirmation, and Looss's observation must first be proved erroneous.

From the foregoing description it will be apparent that *Brachycladium oblongum* (Brn.) and *Br. palliatum* (Lss.) cannot be confused, for the two species differ not only in their coarser anatomy, as Braun has already demonstrated, but also in many of the finer details. It will also be admitted that *Br. oblongum* does not conform strictly to the type of *Br. palliatum*, but differs from it in such important features as the structure of the ductus ejaculatorius, the reduced condition of the receptaculum seminis, with the presence of a receptaculum seminis uterinum, and possibly most important of all, the shape of the ova. How far any or all of these divergences are due to an error of observation on Looss's part, particularly as regards the ova, it is impossible to say, but assuming them to be correct there can be little doubt that the two species cannot be included in the same genus. Not only so but the condition of the ova would render *Br. palliatum* Lss. an atypical member of the BRACHYCLADINÆ according to Odhner's diagnosis. On the other hand, *Br. oblongum* conforms to the sub-family definition, but is an aberrant member of the genus *Brachycladium* Lss. (type *Br. palliatum* Lss.). At present it seems best to await fuller knowledge of Poirier's species before pronouncing judgment on the matter.

Sub-family ALLOCREADINÆ (Lss. 1899).

Genus *Lebouria* n. g.

Lebouria idonea n. sp., Pl. 9, figs. 9-12.

This form occurred very regularly and always in large numbers throughout the intestine of *Anarrhichas lupus*. It also occurred less frequently in the stomach. It is easily

seen in the midst of the intestinal contents on account of its distinctly yellow colour.

It is of medium size, 1·5–2·5 mm. long, with a maximum breadth of ·7–1·0 mm. Thickness ·2 mm, fairly uniform except at the ventral sucker, where it reaches ·3 mm. Its breadth is therefore nearly half its length, so that it is broader than most other ALLOCREADIINÆ. The shape is oval, slightly attenuated anteriorly, considerably flattened dorso-ventrally. One example showed a curious deformity. In the posterior half of the body on the level of the testis there was a large indentation on the right side, an appearance as if a part had been bitten out. The specimen was uninjured, and although it was alive and moving actively the deformity still remained. It may be regarded as a pathological curiosity.

The smallest mature specimen obtained measured 1·58 mm. The largest immature specimen had the same length, and many were found without ova just about this size or a trifle smaller, so that this probably represents the size at which ova begin to appear, and may be taken as the minimum length of adult specimens. The smallest immature example measured 1·23 mm.

The cuticle is about ·004 mm. thick and entirely without spines. True unicellular cutaneous glands, of the same kind as already described in *Stephanophiala laureata*, are present in the anterior part of the body, chiefly laterally. They are large, oval cells with distinct nuclei, and with their long axis in the length of the body, but they differ in appearance from the corresponding cells in *St. laureata*. They present a very distinct outline with pale, scanty, granular contents. This may be due to a difference in the condition of the cells or possibly to a slight difference in staining.

The subcutaneous muscular system is as usual. The circular fibres measure ·0005 mm. and are separated by spaces of ·004 mm. The longitudinal fibres measure ·0007 and are separated by ·0135 mm. The diagonal fibres measure ·001 mm. with spaces of ·045–·055 mm. The muscles throughout the rest of the body are well developed, especially

in the neighbourhood of the ventral sucker. The myoblasts of the subcutaneous muscles are numerous, and arranged in groups. Their nuclei measure $\cdot 004$ – $\cdot 005$ mm.

The oral sucker is subterminal and nearly round, measuring $\cdot 17$ – $\cdot 28$ mm. The ventral sucker is situated very nearly in the middle of the body and is transversely oval. Its long axis measures $\cdot 41$ – $\cdot 58$ mm., its short axis $\cdot 31$ – $\cdot 48$ mm. It is thus about twice the size of the oral sucker, and the transverse diameters of the suckers are respectively about one tenth and two ninths of the body-length. The internal structure of the suckers offers nothing peculiar.

The pre-pharynx is distinct and rather wide. The pharynx is of comparatively large size, measuring $\cdot 15$ – $\cdot 21$ mm. in diameter. It is nearly globular, but the breadth is slightly greater than the length. It is proportionately much larger than it is in any other member of the ALLOCREADINÆ. The œsophagus is about as long as, or a little shorter or longer than, the pharynx ($\cdot 1$ – $\cdot 18$ mm.). In section it is transversely oval. The intestinal bifurcation occurs almost midway between the suckers. The diverticula are fairly wide ($\cdot 04$ – $\cdot 07$ mm. in a large specimen). They pass round the sides of the ventral sucker and, taking up a dorsal position, extend nearly to the posterior end of the body. The œsophagus is sharply differentiated from the diverticula, not only by the change in the nature of the lining, but also by a well-marked constriction (fig. 10). The bifurcation is of the nature of a dilated sac formed by the fusion of the initial parts of the diverticula. In appearance it rather simulates a small ventricle. The pharynx and œsophagus are lined with a cuticular membrane; that of the former is probably true cuticle, that of the latter is transformed epithelium. In any case the two are not identical, for the pharyngeal lining is thicker and stains more deeply than the œsophageal. The bifurcation and diverticula are lined throughout by a well-developed epithelium, the nuclei of which are round and measure $\cdot 0052$ mm. Numerous hair-like processes are given off from the cells. The musculature of the alimentary canal

is well developed. The fibres of the œsophagus are stouter and more closely set than those of the diverticula. They have a diameter of about $\cdot 0015$ mm., and are separated by about the same distance or slightly more in the case of the longitudinal fibres. Those of the diverticula measure $\cdot 0004$ mm. with spaces of $\cdot 0035$ mm. in the case of the annular fibres, and $\cdot 0075$ mm. in the case of the longitudinal fibres. These figures of course are only approximate, for there is great variation with the state of contraction. A moderate number of circum-œsophageal cells (myoblasts) are present.

The excretory system is of simple type. There is a small undivided vesicle not extending further forward than the anterior testis and opening by a pore at the posterior end of the body. From the vesicle two narrow, transversely oval, collecting tubes are given off. They pass outwards to lie close to the outer side of the intestinal diverticula. Their course thereafter was not followed.

The testes are two transversely-oval bodies of rather irregular outline lying midway between the ventral sucker and the posterior end of the body. They are very close together, practically contiguous, and very often somewhat obliquely set, one behind the other. The anterior testis is displaced to the left side, while the posterior is nearly median or a little to the right. In many specimens they were found directly tandem, but no other difference could be found in those specimens, so they are probably only a variety. The testes are very nearly equal in size, the posterior being, if anything, a little larger. The long diameter measures $\cdot 31$ – $\cdot 37$ mm., the short diameter $\cdot 19$ – $\cdot 22$ mm. The vasa deferentia arise from their anterior surface and pass forwards ventral to the shell-gland complex as two almost straight lines towards the vesicula seminalis.

The cirrus-pouch (fig. 11) is of no great size, but it appears shorter than it actually is, owing to the backward displacement of the ventral sucker. It extends only a short distance beyond the anterior border of the sucker. Its total length is about $\cdot 4$ mm. The narrow anterior part, which has a terminal

diameter of about .03 mm., usually runs straight back from the genital aperture, while the thicker part is bent on this either to the right or left. The diameter of the latter reaches a maximum of .09 mm. Its wall consists of the usual double layer of muscle-fibres, which have a diameter of about .0009 mm. and are closely set. The vesicula seminalis is remarkable for the extent to which it is convoluted (fig. 11), exceeding that of *Podocotyle atomon* (Rud.) in this respect. It appears, however, to have a fairly definite configuration. From the end of the cirrus-pouch it passes forwards in a curve towards the right. It then turns completely on itself and bends back in an S-shaped course nearly to the posterior end of the pouch, where it again turns and runs forward almost parallel to the first part. It then passes into the ductus ejaculatorius, which almost immediately bends backward, describes a turn, passes forwards again, only to describe another complete turn, after which it runs forwards in a sinuous manner, but without further convolutions. The diameter of the vesicula seminalis is .04 mm.; that of the ductus is about .015 mm.

As in *Podocotyle atomon* (Rud.) no well-differentiated *pars prostatica* occurs on the ductus. Around the initial part of the ductus, however, there is a considerable number of large cells which occupy the usual position of the prostatic cells. They differ from the corresponding cells in *Stephanophiala laureata*, *Brachycladium oblongum* and other species which I have examined. The cell body appears to be very tenuous and almost invisible, but the nucleus and nucleolus stain very brightly. The nucleus is large and round, measuring .0068 mm., the nucleolus .0018 mm. They certainly do not lend the impression of being gland-cells, but from their position they can hardly be otherwise. They are quite distinct from the "Begleitzellen" or myoblasts which are present in large numbers around the anterior two thirds of the ductus. The latter are much smaller cells, staining deeply and resembling the peri-vaginal cells.

The genital aperture is situated midway between the

suckers, just over the intestinal bifurcation. In most specimens it is approximately median, but in many it is displaced a little to the left side, and in a few a little to the right. The normal position may therefore be regarded as median, but with variations, and this probably indicates one of the initial stages of the transformation to a distinctly lateral position as in *Podocotyle*. The only other members of the ALLOCREADINÆ, as at present recognised, which have a lateral genital aperture, are *A. tumidulum* (Rud.) and *A. angusticolle* (Hausmann), in the former of which the aperture is well to the left, in the latter only slightly. In all the other species it is median, and it is surprising that more forms have not been described showing intermediate stages of this displacement, for it is evident that it can only have occurred by a gradual series of changes. It would be interesting to discover the reason of the transposition and why it is to the left.

A small genital sinus is present, into which the male duct opens on the right, the female on the left.

The ovary is situated immediately in front and to the right side of the anterior testis, with which it is contiguous. It also lies close beside the intestinal diverticulum, and is separated from the ventral sucker by a distance equal to the diameter of the ovary. It is a round, almost globular body, with entire margin and measures $\cdot 16$ – $\cdot 17$ mm. in diameter. The ovarian cells are largest anteriorly, being about $\cdot 01$ mm. in size. The oviduct arises from the anterior dorsal surface of the ovary, and passes in a curved course towards the receptaculum seminis. This is a large oval sac, measuring $\cdot 1$ – $\cdot 16$ mm., attached to the oviduct without the intervention of a pedicle. Laurer's canal arises directly from the receptaculum and runs almost straight in towards the middle line of the body. The exact position of its opening could not be ascertained. The receptaculum lies dorsal to the ovary, but its position is variable, being sometimes in front of the ovary, sometimes behind it. In fig. 12 it is shown in the latter position. From the receptaculum the oviduct passes across the ovary to the shell-gland, before entering which it is

joined by the common yolk-duct. The shell-gland lies close to the left side of the ovary and receptaculum. It is a small, compact structure with cells measuring $\cdot 015$ mm. and nuclei $\cdot 0058$ mm. The cells have a distinct staining reaction, taking on a uniform dull red or reddish-purple with hæmalum-eosin.

The small yolk-reservoir lies dorsally in the middle line of the body just in front of the anterior testis. The yolk-glands are lateral, extending from the pharynx to the posterior end of the body, where they unite behind the testes. Anteriorly a few follicles find their way across the middle line under the dorsal surface, but none ventrally. Posteriorly the follicles lie under both the dorsal and ventral surfaces and surround the intestinal diverticula. They are of moderate size, comprising twelve to twenty cells, which measure about $\cdot 023$ mm. in diameter, and have large ($\cdot 006$ mm.), round, central nuclei.

The uterus is limited in extent, being confined between the ovary and the posterior border of the ventral sucker, but also filling up the space between the sucker and the left intestinal diverticulum. The vagina is shorter than the cirrus-pouch, lying to the left of it and slightly dorsal. Its diameter diminishes from $\cdot 035$ mm. to $\cdot 023$ mm. It has the usual muscular structure with numerous accompanying myoblasts.

The ova are not numerous (about sixty). They are light yellow, with a very thin shell. Their shape is broadly oval, but at the anopercular pole there is a small excrescence of the shell forming a small knob-like projection.¹ This is of variable size and is sometimes absent. It might be regarded as indicating an initial stage towards the filamented condition of the ova in *Helicometra*. They measure $\cdot 0715$ – $\cdot 0746$ mm. in length by $\cdot 039$ – $\cdot 0435$ mm. in breadth. Average $\cdot 073$ by $\cdot 041$ mm.

From *Anarrhichas lupus* six species of Distomids have at one time or another been recorded. The first of these are two species mentioned by Rathke² and named by Rudolphi³ *Distomum incisum* and *D. anarrhichæ lupi*, found re-

¹ For directing my attention to this I am obliged to Miss Lebour.

² 'Dansk. Selsk-Skrift,' v (1), (1799), p. 70, pl. 2, figs. 3, a, b.

³ 'Entoz. Hist. Nat.,' ii (1), (1809), pp. 361, 435.

spectively in the intestine and the stomach. The description of both is extremely scanty and hardly admits of positive identification. *D. incisum*, it is true, was regarded as identical with *D. fellis* Olsson by Stossich, but this is denied by Jacoby.¹ It is almost certain that neither species is identical with *Lebouria idonea*. *Distomum atomum* Rud. is noted by von Linstow² under *Anarrhichas lupus*, but in the reference³ it is only recorded from *Platessa flesus*. The peculiar condition of the ova which von Linstow describes distinguishes his specimens from *Podocotyle atomum* (Rud.), and renders it not at all improbable that they were really specimens of *Lebouria idonea*. The first mention of this actual species is by Miss Lebour,⁴ who has kindly allowed me to describe it in consideration of a previous communication of its discovery which I had made. It is not the only species which occurs frequently in the intestine of *Anarrhichas lupus*, for in invariable association with it, but usually in the lower parts of the intestine, I have met the much smaller form *Zoogonus rubellus* (Olsson). This is the first record of the latter species from the cat-fish, and also the first time it has been met with in British waters, yet it is a constant parasite of the cat-fish in this locality.

It remains to differentiate *Lebouria idonea* from already recognised members of the sub-family ALLOCREADINÆ. From *Podocotyle* (Duj.) it is easily distinguished by the median position of the genital aperture; from *Helicometra* Odhner by the non-filamented condition of the ova, and from *Allocreadium* Lss. sens. str., by the oblique position of the testes, the backward situation of the ventral sucker, and the absence of distinct pars prostatica. Amongst the unclassified forms it appears to bear most resemblance to *Distomum tumidulum* Rud.,⁵ but that species is more elongated, has a

¹ 'Arch. f. Naturg.,' lxvi, p. 13.

² 'Compend. d. Helminth. Nachtrag,' p. 82.

³ 'Arch. f. Naturg.,' xlv, p. 225.

⁴ 'Northumberland Sea Fisheries Report for 1907,' p. 16.

⁵ Odhner, "Revision einiger Arten der Distomungattung *Allocreadium* Lss.," in 'Zool. Jahrb. Syst.,' xiv, pp. 503-505.

distinctly lateral genital aperture,¹ smaller ova, and round testes. Were it not for the lateral genital aperture it might readily enough be included in the genus *Lebouria*, and it is certainly more nearly related to this genus than it is to *Podocotyle*. This affinity may be indicated by re-naming the species (*Lebouria*) *tumidula* Rud. From the other *ALLOCREADIINÆ*, *Lebouria idonea* is much more easily distinguished.

In proposing this species as the type of a new genus I have been influenced by the fact that it is impossible to include it under the genus *Allocreadium* Lss., and therefore its description as an *Allocreadium* sp. would have been erroneous. Moreover, it appears to be the representative of a group with features quite as well defined as those of *Helicometra* and *Podocotyle*, and further, it is not an isolated species, for in an American form described by Linton² from *Bairdiella chrysura* we have what must be regarded as an additional number of the genus. Linton's work is, unfortunately for our present purpose, more of a bionomic than morphological character, so that his treatment of the species is not so exact as we might have desired. His figures, however, give a fairly good idea of the form, and its close resemblance to *Lebouria idonea* is not difficult to make out. We note the expanded shape of the body, the backward position of the ventral sucker with the intestinal bifurcation far in front of it, the oblique, or sometimes median, position of the testes, the round ovary, extensive yolk-glands, limited uterus, short cirrus pouch, and median genital aperture. With regard to the position of the genital aperture it is shown in fig. 168 midway between the ventral sucker and the intestinal bifurcation, but from the forward position of the ova it may possibly be found to be somewhat

¹ I have since found in *Labrus bergylta* a Distomid which I am inclined to identify as *Distomum tumidulum*, and in it the genital aperture is median or very slightly displaced.

² 'Bull. Bureau U.S. Fisheries,' xxiv (1904), p. 389, pl. xxiii, figs. 168-170.

nearer the bifurcation. Linton was somewhat doubtful of the identity of this species and hesitated to assign to it a specific name. I therefore propose for it the name *Lebouria obducta* n. sp., and add the following short description.

Length about .8 mm.; breadth half the length; oral sucker .12 mm.; ventral sucker oval, a little in front of the middle of the body, diameter .17 mm. (this is probably the short diameter, for in Linton's figures the transverse diameter is always at least twice that of the oral sucker). Pharynx, length .06 mm.; œsophagus short; genital aperture a little behind intestinal bifurcation. Testes much larger than those of *Lebouria idonea*, irregularly shaped, in posterior third of body. Cirrus-pouch short, extending a little beyond the anterior border of the ventral sucker. Ovary transversely oval, on a level with anterior testis or a little in front, on right side. Yolk-glands only reaching intestinal bifurcation, not uniting in front but filling up space behind testes. Ova few (five to twenty) measuring .063 by .035 mm.

From a comparison of these two species the genus *Lebouria* may be defined as follows:

Small ALLOCREADIINÆ with broad, flat, oval body. Ventral sucker oval, larger than oral sucker, situated about the middle of the body or a little in front of it. Œsophagus short, intestinal bifurcation midway between suckers. Excretory vesicle simple, short. Genital aperture median or slightly displaced, near the intestinal bifurcation. Testes of irregular shape, oblique or tandem, in the middle of the post-acetabular region. Cirrus-pouch short, not reaching the middle of the ventral sucker, containing a convoluted vesicula seminalis and ductus ejaculatorius, but lacking a well-differentiated pars prostatica, although prostatic cells are present. Ovary rounded, on the right side, on a level with anterior testis or immediately in front of it. Receptaculum seminis and Laurer's canal present. Yolk-glands extending in front of ventral sucker. Uterus short; ova few, thin-shelled, with small protuberance at the anopercular pole, measuring about .07 by .04 mm.

Habitat.—Stomach and intestine of marine fishes.

Type.—*Lebouria idonea* n. sp.; including also *L. obducta* n. sp.

Mention may be made here of another species which bears some resemblance to *Lebouria idonea*, namely, *Distomum alacre* Lss.¹ from *Labrus maculatus* and other Labridæ. At first sight only comparatively unimportant differences are apparent between the two, such as the slightly lateral position of the genital aperture, the somewhat elongated testes and the rather larger ova. The structure of the terminal part of the male genital duct, however, is different from that not only in *Lebouria* but also in other ALLOCREADINÆ, and for that reason, apparently, Looss has hesitated in assigning to this species a systematic position.²

Genus *Podocotyle* (Duj.) Odhn., 1904.

= *Sinistroporus* Stafford, 1904.

Podocotyle atomon (Rud.) Pl. 10, fig 28.

= *Distomum vitellusum* Linton. Johnstone, 'Report Lancashire Sea Fisheries,' for 1906, xxi (1907), pp 182-185, fig. 15.

For the opportunity of examining these specimens I am indebted to my friend Mr. Johnstone, who kindly put them at my disposal. Their resemblance to *Distomum vitellusum* Linton is not borne out on close examination, and they have proved to be in reality a species of *Podocotyle*, most probably *P. atomon* (R.). At first sight I was inclined to regard them as distinct from the latter species. The unusually cylindrical shape of the body, the excessive

¹ 'Centralbl. fur Bakter.,' 1st Abth., xxix, pp. 401-402, fig. 2.

² In a paper by Stossich (in 'Boll. Soc. Adriat.,' xxii, p. 211-217), which has not yet found its way into the London libraries and which I have therefore not had an opportunity of seeing, *Distomum alacre* Lss. is included in the genus *Allocreadium* (not sens. strict). If this species be really a member of the ALLOCREADINÆ it must occupy a place very near the genus *Lebouria*.

prominence of the ventral sucker and the conspicuousness of the yolk-ducts were features unfamiliar to me in my previous experience of the species. As a matter of fact, however, they are due to the method (in fresh water) which Johnstone employed in killing his specimens. I have since found *Podocotyle atomon* in various species of fish, and tried the experiment of killing some in fresh water, in which they assumed the same shape and appearance as Johnstone's specimens.

One of the most striking features of Johnstone's specimens was the distinctness not only of the yolk-ducts but also of the yolk-glands themselves. On that account their disposition could be easily observed, and this is shown very well in Johnstone's figure. One point to which he makes no reference is the occurrence, in about every third specimen, of an asymmetrical group of follicles in front of the ventral sucker on the right side. This had not the linear arrangement of the other follicles, but was more dendritic, and from it a separate duct passed down to join the longitudinal duct. This asymmetrical group was never observed on the left side. I have since collected a number of specimens showing the same characteristic but differing in no other respect from *Podocotyle atomon*. It seems to indicate what might be regarded as a distinct variety, and for this I propose the provisional name *P. atomon* var. *dispar*.

During a recent visit to the Firth of Clyde, I have obtained *Podocotyle atomon* from numerous species of fish, *Gadus virens*, *G. pollachius*, *Pleuronectes flesus*, *Pl. platessa*, *Cottus scorpius*, *C. bubalis* and *Centronotus gunnellus*, and have been struck with the remarkable diversity of form which it presents. It displays variation in the extent of the yolk-glands, the length of the cirrus-pouch, the shape and situation of the testes, and, in particular, the size of the ova. I have to add *Gastræa spinachia* (fifteen-spined stickleback) as an additional host on the east coast, and in the specimens from this host the ova were exceptionally large, reaching a length of nearly .1 mm. This Trematode, if all the specimens be really identical, is very

widely distributed, it having now been recorded from thirteen different hosts in this country.

On the classification of the ALLOCREADIINÆ.

We are now in a position to recognise, at least, four well-defined groups of the ALLOCREADIINÆ, namely, the genera *Allocreadium* Lss., sens strict., *Helicometra* Odhn., *Podocotyle* (Duj.) Odhn. and *Lebouria* Mihi. The types of other groups have been indicated by Odhner,¹ and he has also constructed a key for the diagnosis of several species.² In all probability the ultimate division of the sub-family will proceed along the lines he has laid down, with certain modifications. The imperfect knowledge which we as yet possess of the internal structure of many of these forms renders premature any attempt at a complete classification of the sub-family, and we must be content for the present with speculations. On the other hand, it is advisable in the interests of systematic work, that as many forms as are sufficiently well known should be assigned their true generic place, and on that account I venture to propose, as the types of two new genera, two species which have already been considered by Odhner as probable generic types, and of which I have personal knowledge,³ namely, *Distomum labracis* Dujardin and *Dist. genu* Rud. Both species have been re-described by Odhner,⁴ and the first also by Johnstone.⁵ I propose *Dist. labracis* Duj. as the type of the new genus *Cainocreadium* with the following provisional definition:

Large ALLOCREADIINÆ with extended, flattened body.

¹ "Die Trematoden des arktischen Gebietes"; in 'Fauna Arctica,' iv, p. 327.

² "Revision einiger Arten der Gattung Allocreadiinæ," in 'Zool. Jahrb.,' syst. xiv, p. 516.

³ Thanks in the first instance to my friend Mr. Johnstone, who sent me his specimens of *Allocreadium labracis* Duj.

⁴ 'Zool. Jahrb.,' syst. xiv, pp. 496-499 and 514-515, pl. xxxiii, figs. 3 and 11.

⁵ 'Trans. Biological Soc.,' Liverpool, xxii (1908), pp. 44-53, pl. iii.

Ventral sucker near the middle of the body, globular or slightly oval and not projecting much. Œsophagus short, bifurcation far in front of ventral sucker. Excretory vesicle simple, short. Genital aperture median, not far behind intestinal bifurcation. Cirrus-pouch long and slender, not extending beyond ventral sucker. Vesicula seminalis convoluted, ductus ejaculatorius long; true pars prostatica absent, but prostatic cells present. Ovary on right side, immediately in front of testes, distinctly trilobate. Receptaculum seminis large, giving off Laurer's canal directly. Yolk-glands extensive, filling up considerable part of neck. Yolk-follicles arranged peripherally. Ova without filaments, very variable in size, $\cdot 07$ – $\cdot 10$ mm. by $\cdot 04$ – $\cdot 06$ mm.

For Dist. genu Rud. I propose the new generic name *Peracreadium*, with the following definition :

Small to medium sized ALLOCREADINÆ, with elongated ovate, slightly flattened body. Ventral sucker not very prominent, situated about the end of the anterior third of the body. Œsophagus short, bifurcation midway between suckers. Excretory vesicle simple. Genital aperture median, at intestinal bifurcation. Cirrus-pouch very long and slender, extending as far back as the level of the ovary (= about one third of the body length). Vesicula seminalis convoluted; ductus ejaculatorius long; distinct pars prostatica present. Ovary globular, with entire margin, situated a little to the right side of the middle line, immediately in front of the testes or separated from them by a small part of the uterus. Testes usually transversely oval, with entire margin, situated about the middle of the post-acetabular region. Yolk-glands extensive, occupying considerable part of neck and filling up posterior part of body. Ova without filaments, very variable in size, $\cdot 07$ – $\cdot 10$ mm. by $\cdot 03$ – $\cdot 06$ mm.

Habitat.—LABRIDÆ, so far as known.

Type.—*Peracreadium* genu (Rud.). Also *P. commune* (Olss).

Between these two new genera the main differences consist in (1) the position of the ventral sucker; (2) the shape of

A Table showing the Chief Comparative Differences between the Six Genera of ALLOCREADINÆ.

	Helicometra.	Podocotyle.	Allocreadium.	Lebouria.	Cainocreadium.	Peracreadium.
Size	2-3 mm.	1.5-4.5 mm.	2-5 mm.	1-3 mm.	2-10 mm.	1-3 mm.
Shape	Ovate, flattened	Elongated, sub-cylindrical	Elongated, flattened	Ovate, flattened	Elongated, flattened	Elongated, ovate, not much flattened
Length of neck	$\frac{1}{3}$ or slightly more	$\frac{1}{7}$ - $\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{3}$
Genital aperture						
Cirrus-pouch	Median To centre of ventral sucker	Lateral More or less beyond sucker	Median Not beyond centre of sucker	Median Not beyond centre of sucker	Median Not beyond sucker	Median As far back as ovary
Pars prostatica	Present	Absent	Present	Absent	Absent	Present
Ovary	Multilobate	Trilobate	Round	Round	Trilobate	Round
Testes	Irregular	Round or oval	Rounded	Transverse oval	Rounded	Transverse oval
Yolk-glands	In neck	Not in neck	Not in neck	In neck	Much in neck	Much in neck
Esophagus	Short to medium	Short	Long	Short	Short	Short
Ova.	Filamented	Without filaments	Without filaments	Without filaments	Without filaments	Without filaments
Size of ova	.07-.085 mm.	.07-.095 mm.	.09 mm.	.06-.075 mm.	.07-10 mm.	.07-10 mm.
Type	H. pulchella (R.)	P. atonon (R.)	.06 mm. A. isoporum Lss.	.035-.045 mm. L. idonea Nic.	.04-.06 mm. C. labracis (Duj.)	.03-.06 mm. P. genu (R.)
Other species	H. fasciata (R.)	P. reflexa (Crepl.)	(A.) transversale Rud.	L. obducta Nic.		P. commune (Olss.)
	H. sinuata (R.)	P. olssonii Odh.		(L.) tumidula R.		
	H. mutabilis Stoss.					

the ovary and possibly also of the testes; (3) the length of the cirrus-pouch; and (4) the presence of distinct pars prostatica.

Of the remaining unclassified forms nearly related to the ALLOCREADINÆ I shall refer here only to those described by Stossich¹ and Linton.² Of these *Distomum umbrinæ* Stossich, as already indicated by Odhner, probably represents a distinct genus type, the characteristics of which are: (1) the rather stout, broad body; (2) the very short (or absent) œsophagus; (3) the long cirrus-pouch reaching the ovary; (4) small round testes; (5) globular ovary (on left?); (6) median genital aperture; (7) yolk-glands confined behind ventral sucker. These form a group of features sufficiently important to exclude the species from any of the already formed genera. *Distomum obovatum* Molin and *D. mormyri* Stoss. bear much resemblance to each other and to *D. umbrinæ* Stoss. They differ from the latter, however, in the flatter body, the apparently lateral position of the genital aperture, the yolk-glands extending into the neck, the ovary on the right side and the transverse oval shape of the testes. These three species are probably more nearly related to *Peracreadium* than to any other genus. Another species which appears to approach those in some degree is that depicted by Linton in fig. 165 and found in *Sphenoides maculatus*. It is characterised by a very small ventral sucker, a cirrus-pouch just extending beyond the sucker, transverse testes and yolk-glands confined behind ventral sucker. *Distomum scorpænæ* Rud. Stoss. (I, p. 158, fig. 20) is an extremely doubtful member of the sub-family. It is characterised by the peculiar shape of the body³ and of the œsophagus. Otherwise it bears a distinct resemblance to *Podocotyle*, but the ovary is globular and the cirrus-pouch may prove to be of unique structure. *Dist. fasciatum* Rud.

¹ "Brani Elmint. Tergest.," in 'Boll. Soc. Adriat.,' ix, No. 1 (1885), pp. 158-161, pl. iv-vi; and No. 2 (1886), pp. 44-49, pl. viii.

² "Parasites of Fishes of Beaufort, North Carolina," in 'Bull. Bur. Fisheries, U.S.A.,' xxiv (1904), pp. 321-428, pl. xxii-xxiv, xxviii.

³ This peculiar shape can, however, often be observed in *Podocotyle atomon*.

Stoss (I, p. 160, fig. 25) is regarded by Odhner¹ as wrongly identified. The long cirrus-pouch brings this species into relation with *Peracreadium*. In other respects it also agrees closely with this genus.²

Distomum vitellosum Linton (p. 335, figs. 176–178) is a species which shows some affinity to the ALLOCREADINÆ, but is distinguished by the peculiar structure of the ventral sucker. Linton has described this species from numerous fish, but his descriptions are so conflicting that it is either a very variable species or it is a combination of two or more distinct species. It is undoubtedly a genus type, but its structure is not sufficiently well known for it to be definitely included amongst the ALLOCREADINÆ.

One other species may here be mentioned, namely, *Distomum globiporum* Rud. Linton (p. 334, figs. 159, 173, 198) from *Leiostomus xanthurus* and other fish. This species is quite incorrectly identified. It belongs to the subfamily LEPOCREADINÆ Odhn., but to which genus it is difficult to say. It seems, however, more nearly related to *Lepocreadium* than to *Lepodora* (*Lepidapedon*), and it is not identical with any of the European forms. The specimens which Linton obtained certainly do not all belong to the same species. Those from *Fundulus majalis* (p. 356, fig. 159) are small with equal suckers, round testes, and apparently without spines, and with the genital aperture immediately in front of ventral sucker. Those from *Orthopristis chrysopterus* (p. 378) are twice as large, with smaller ova, ventral sucker half as large again as oral sucker. Genital aperture immediately behind pharynx, and vitellaria extending forward to the middle of the neck. Those from *Leiostomus xanthurus* (pp. 393–394, figs. 173, 198) are a mixed lot, some with spines, others without. The

¹ "Revision der Gattung *Allocreadium*," in 'Zool. Jahrb.,' syst. xiv, p. 486.

² In Stossich's latest paper (see p. 61, note 2) on these species, *Allocreadium characis* Stoss., *A. mormyri*, and *A. umbrinæ* are dealt with. A new species, *A. dubium*, is added. Whether he deals with *Distomum fasciatum* Rud. or not I am unaware.

ventral sucker is distinctly larger than the oral sucker, the genital aperture is immediately in front of the ventral sucker, testes lobed, and ova measure about $\cdot 10$ by $\cdot 05$ – $\cdot 06$ mm. The specimens which Linton found on August 10th, 17th and 30th (pp. 393–394, figs. 198–199) appear to represent a fairly distinct species, the characters of which may be summed up as follows: Length 3 mm.; breadth 1.25 mm. Oral sucker $\cdot 4$ mm. in diameter; ventral sucker $\cdot 5$ mm. Cuticle covered with scale-like spines on dorsal surface and anterior ventral surface. Pharynx $\cdot 12$ mm. Pre-pharynx and œsophagus each about same length as pharynx. Genital aperture median, immediately in front of ventral sucker. Cirrus-pouch extending back to anterior border of first testis. Testes median, tandem, slightly lobed, situated near the middle of the post-acetabular region. Ovary sub-globular not far behind ventral sucker, separated from testes by the end of the cirrus-pouch, median or slightly to right side. Yolk-glands lateral to outer side of intestinal diverticula, filling space behind testes, not extending in front of ventral sucker. Ova not numerous, measuring $\cdot 10$ – $\cdot 12$ by $\cdot 05$ – $\cdot 06$ mm. To this species the provisional name (*Lepocreadium*) *serospinosum* sp. inquir (= *Distomum globiporum* Rud. Linton, 1904, e.p.) may be given.

Sub-family FELLODISTOMINÆ, n. sub-fam.

Genus *Fellodistomum* (Stafford 1904).

- Fellodistomum fellis* (Olsson, 1868). Pl. 9, figs. 13, 14.
Distoma fellis Olsson, "Entozoa, iakt. Skand. hafsfisk,"
 in 'Lunds Univ. Årssk.,' iv (8), pp. 44–46, pl. v., fig. 94,
 A, B.
Distomum fellis Olsson, Stossich, "I. Distomi dei Pesci,"
 in 'Progr. d. Ginnasio comm. super. Trieste,' 1886, p.
 24.
Distomum fellis Olsson, Jacoby, "Beitr. z. Kenntnis. enig.
 Distomen," in 'Arch. f. Naturg.,' lxvi (1) (1900), pp.
 12–16, pl. ii, figs. 8–12.

? *Fellodistomum incisum* (Rud.) Stafford, "Trematodes from Canadian Fishes," in 'Zool. Anzeig.,' xxvii (1904), p. 486.

This species has already been recorded from the east coast of Britain by Miss Lebour. It is a remarkably frequent parasite of the cat-fish (*Anarrhichas lupus*) being met with in almost every specimen, young and old. Its habitat is exclusively the gall-bladder. Fifty examples, at least, can usually be obtained from one host.

On the question of the identity of this species with two species found by Rathke¹ in *Anarrhichas lupus* and named by Rudolphi,² several authors have ventured an opinion. Van Benedin³ considered *D. fellis* Olss. synonymous with *D. incisum* Rud. Stossich also evidently considered the two species identical, but retained Olsson's name. Jacoby repudiated the possibility of such identity. Stafford, leaving his reasons unexplained, adopts Rudolphi's name, and makes *Dist. incisum* Rud. the type of his genus *Fellodistomum*. How he identifies the species which he describes with Rudolphi's species is to me not very apparent. It has little in common with Rudolphi's description.

As regards *D. incisum* Rud. my opinion coincides with that of Jacoby. By no legitimate means can the form figured by Rathke and described by Rudolphi be made to agree with *D. fellis* Olsson. On the other hand, Rathke's second species, which Rudolphi⁴ provisionally named *Dist. anarrhichæ lupi*, bears an undeniable resemblance to *Dist. fellis*.⁵ The only description of this form, beyond its habitat and size, is contained in Rathke's words "*corpore elongato carneo, apertura dorsali rotunda annulo luteo*

¹ 'Dansk. Naturhist.-Selsk. Skrivt.,' v (1799), p. 70, pl. ii, fig. 3.

² 'Entoz. Hist.,' ii (1), p. 361.

³ 'Mém. Acad. Roy. Belg.,' xxxviii (1870), p. 48.

⁴ 'Entoz. Hist.,' ii (1), p. 435.

⁵ Olsson undoubtedly recognised this, for he marks *D. anarrhichæ lupi* as doubtfully identical with *D. fellis*. At the same time he completely ignores such a possibility in the case of *D. incisum* Rud.

cincta," but these words describe exactly the chief features which struck me when I removed my first specimens from the gall-bladder of their host. The thick, fleshy body extended vigorously towards both ends, the large knob-like ventral sucker (apertura dorsali), pale pink or flesh-like in colour and surrounded by a strikingly bright yellow ring contrasting strongly with the dull greenish hue of the rest of the body. These appearances are lost on preservation, and it was on this account, I believe, that Jacoby, who examined preserved material, was not impressed with Rathke's description. A most potent objection to the identification of *Dist. anarrhichæ* with *D. fellis* is the difference in habitat, for Rathke's specimens occurred in the stomach. Various hypotheses might be advanced to meet this difficulty, but it makes no difference to the matter in hand, for, as Looss has explained, such names as *D. anarrhichæ lupi* were not intended by Rudolphi as specific names, so that the name *D. fellis* Olsson remains good.

With regard to Stafford's specimens, the ovary is described on the left side and the testes as large and spherical. According to Jacoby's observations, which I confirm, the ovary always lies on the right side and the testes are obliquely oval in *Distomum fellis*. This renders it doubtful if Stafford's specimens are really identical with *D. fellis*, but it is possible that Stafford's statements may be due to a slip of the pen.

My adult specimens measure 2.5-3.3 mm. in length and 1.1-1.6 mm. in breadth. The normal breadth seems therefore to be about half the length, but the animal is capable of considerable extension. In the contracted state the breadth may almost equal the length and the animal becomes nearly globular. The shape in the quiescent state is most probably that represented by Jacoby. The body is thick and nearly opaque.

Numerous immature specimens were found, and these measured 1.1-2.5 mm., so that in this species maturity is reached at a size of about 2.5 mm.

As already mentioned the large ventral sucker surrounded by a yellow ring is the most prominent feature seen on ventral view. On the dorsal aspect the two intestinal diverticula show up very conspicuously by reason of their dark green contents.

The oral sucker is sub-terminal and globular, measuring .40-.45 mm. The ventral sucker is also globular, situated a little behind the centre of the body. Its aperture may be either circular, transverse or diamond-shaped. Its diameter is .9-1.0 mm., therefore about twice that of the oral sucker. Although of such great size, it is deeply sunk into the body and does not project much above the surface.

The histological structure of the suckers, especially the ventral sucker, presents many features of interest, and here my observations do not entirely agree with those of Jacoby. There are three chief varieties of cells: First, the large myoblasts (fig. 14, G Z). These are extremely few, not more than two or three appearing in any one section. They are very large cells, measuring .03-.05 mm., and, as Jacoby observed, the cell-body stains homogeneously deep red. The nucleus is central and oval in shape, measuring about .02 by .015 mm. It is traversed by a well-defined chromatin network, in the midst of which is situated a globular nucleolus, the diameter of which is .007 mm. This type of cell differs from the myoblasts usually met with in other species, or at any rate in the species which I have hitherto examined, chiefly in the denser character of the nucleus and its shape.

These cells, however, have the same situation as the myoblasts in other species, namely, the median zone, midway between the outer and inner limiting membranes of the sucker.

The second variety of cell (fig. 14, D Z S) is that identified by Jacoby as the cells which Schwarze¹ considered as "*Reste der ursprünglichen Bildungszellen.*" These are the cells marked "G Z" in Jacoby's figures, and they occur chiefly, if not exclusively, round the aperture of the sucker. They

¹ "Die postembryonale Entwicklung der Trematoden," in 'Zeitsch. f. wiss. Zool,' xliii (1886), p. 54.

are large, rounded, oval cells, measuring $\cdot 045$ by $\cdot 03$ mm. They present a distinct cell wall, loosely granular contents and a small, dense nucleus, measuring $\cdot 006$ mm. The nucleus stains dark red and the cell contents light purple with hæmalum-eosin. Jacoby's opinion of these cells is highly improbable. In reality they appear to be true cutaneous glands. Their marked resemblance both in shape and staining properties to the true subcutaneous glands, which in this species occur not only in front of, but also behind the ventral sucker, cannot be ignored. This, together with their distinctly marginal situation, if not a positive proof of their glandular nature, is at least strong evidence in favour of such a supposition. In addition it should be noted that these cells are smaller and less numerous in the oral sucker and are entirely absent from the pharynx.

The third variety of cell really consists of at least three different kinds. The first of these (fig. 14, p z) is confined to the outer zone, i.e. immediately beneath the outer membrane of the sucker. Their nuclei are small, dense, round bodies, measuring $\cdot 008$ mm. The nucleoli are small and inconspicuous. The cell-body is usually fairly definite and of granular structure. The second kind (fig. 14, m z) occupies the median zone with the myoblasts. Their nuclei are invariably oval, and measure $\cdot 0115$ by $\cdot 008$ mm. They stain lightly and their chromatin granules are arranged peripherally. The nucleoli are always distinct. No trace of cell-body, however, can be detected with ordinary staining methods. The third kind (fig. 14, i z) lies in the inner zone. They are distinctly multipolar, and in a tangential section their fibrils can be seen anastomosing with each other. The cell-body takes on a deeper hue than any of the other cells, almost purple. They measure $\cdot 02$ to $\cdot 03$ mm., and their nuclei, which are small, dense and usually oval, measure $\cdot 007$ by $\cdot 006$ mm.

As to the function of these last three kinds of cells any expression of opinion would be hazardous. Many authors have advanced theories as to their nature, but without any

generally accepted conclusion. There is evidently scope for further investigation on the matter.

The foregoing observations apply mainly to the ventral sucker. In the oral sucker the cells are more closely packed and not so easily differentiated. It is also worth mentioning that in some specimens the marginal cells, to which I have ascribed a glandular function, can hardly be distinguished. These are generally highly contracted specimens. In most cases, however, these cells stand out with striking distinctness and give sections of the sucker an entirely unique appearance.

In spite of their large size the suckers are not particularly muscular. The fibres are widely separated and the interspaces are filled with a large amount of loose tissue. The radial fibres have a diameter of about $\cdot 001$ mm. in the case of the ventral sucker. Two layers of circular fibres are present under both the external and internal surfaces.

The surface of the body is covered with a fairly uniform cuticle, $\cdot 002$ – $\cdot 004$ mm. thick; Jacoby says $\cdot 0113$, but that is certainly too high a figure. There are no spines, but the cuticle is covered throughout its whole extent with curious little rod-like bodies, measuring $\cdot 004$ by $\cdot 001$ mm. (fig. 13, Cu.). These are regularly arranged, close together, and stand out straight from the surface, not sloped backwards in the manner of spines. They are purely superficial outgrowths and do not penetrate the cuticle. It is strange that no mention is made of these by Jacoby, for their appearance is certainly remarkable enough. It may be that they are of transitory occurrence, but they were present in every specimen which I sectioned. The cuticle lining the suckers and the pharynx also shows these rod-like bodies in many specimens.

In the anterior part of the body the cuticle is thrown into numerous small transverse wrinkles. These are not the irregular wrinkles so commonly met with in other species; they are fairly uniform in size, and in longitudinal section present the appearance of a series of regular furrows. Of this, again, no mention is made by Jacoby. In the posterior part of

the body the cuticle does not display the same wrinkling, and it is not present on the dorsal surface of the body. The little rod-like bodies follow the course of these wrinkles, as do also the circular subcutaneous muscle-fibres. The longitudinal muscles, however, do not; their course is straight and it is evidently to their action that the wrinkling is due.

The diameters of the circular, longitudinal and diagonal muscle-fibres are respectively about $\cdot 0015$ mm., $\cdot 003$ mm. and $\cdot 004$ mm., and the spaces separating them are on an average $\cdot 0035$ mm., $\cdot 007$ mm. and $\cdot 008$ mm. in the three cases. The angle made by the diagonal fibres is about 135° . The myoblasts in connection with these fibres are quite as numerous as usual.

The true subcutaneous glands are large and numerous; they occur only on the ventral surface, but they are not confined to the anterior part of the body as is the case in most species. A considerable number are to be found behind the ventral sucker, but they cease about the middle of the post-acetabular region. They measure about $\cdot 045$ by $\cdot 03$ mm., and are rounded, oval in outline with highly granular contents and small eccentric nuclei, $\cdot 007$ mm. in diameter.

Alimentary System.—There is a short pre-pharynx, in the wall of which well-marked circular and longitudinal muscle-fibres are present. The pharynx has a length of $\cdot 16$ – $\cdot 21$ mm.; its breadth is usually somewhat less. It appears to be more muscular than the suckers, for in addition to the usual fibres several equatorial fibres are present. It contains very few cells, and these are almost entirely of the myoblast type, situated in the middle zone, but much smaller than those of the ventral sucker.

There is practically no undivided œsophagus, the bifurcation taking place immediately behind the pharynx, but the initial parts of the diverticula are not lined with intestinal epithelium. Each of these parts is about $\cdot 1$ mm. long and $\cdot 04$ mm. wide. They are lined by a cuticularised membrane. The diverticula are simple wide sacs running nearly the whole length of the body, but terminating at the posterior

end of the testes. Anteriorly their width is about .2 mm. but further back they dilate to .3 mm. They are situated just below the dorsal surface of the body. The intestinal epithelium is exceptionally well marked. It is cubical or columnar in type, but the cells are all of different sizes and shapes, projecting to various degrees into the lumen. Towards the posterior end of the diverticula the cells are usually flatter. It is evident that these cells are capable of considerable pseudopodial movement, for they contain varying amounts of bile which they have ingested (fig. 14, z). The extended cells contain a large amount of bile surrounded by a vacuole. As the bile becomes metabolised and absorbed the cell contracts and the vacuole disappears. Two neighbouring cells may thus present a great contrast, one being highly extended and packed full of bile, the other small, flat and without any trace of bile. From this there can hardly be any doubt that the cells actually engulf the intestinal contents. In their extended state they may reach a length of .04 mm. They possess small round nuclei situated basally. The hair-like or thread-like processes so frequently described in other species are entirely absent in this, and it appears as if the character of the epithelium and the manner of food absorption in this species were different from that in most other species. Here, however, the dark-green colour of the intestinal contents renders its presence within the epithelial cells a matter of easy observation—a fact which is by no means so easy to demonstrate in those species in which the intestinal contents are colourless or nearly so. It appears highly probable that in many cases a certain restricted pseudopodial movement of the epithelial cells does really take place, but it is a quite as well attested fact that in many other cases no such movement occurs, and that the process of assimilation of material, partially metabolised in the lumen of the intestine, is effected by a capillary action of the hair-like processes of the epithelial cells.

Excretory System.—The vesicle is of the Y-shaped type. The main stem is short and almost diamond-shaped, its length

being about .2 mm. and greatest breadth .1 mm. It lies between the two testes and bifurcates at their anterior border. The limbs pass round the sides of the ventral sucker and take up a position immediately ventral to the intestinal diverticula. They run forward as far as the level of the pharynx, and their ends occupy a position about midway between the pharynx and the margins of the body. The whole vesicle, both stem and limbs, is lined by a well-marked epithelium, the individual cells of which in many cases stand out prominently and can be easily observed (figs. 13, 14, Ex.). At other places they are flattened and not so distinct, so that here, again, we are evidently dealing with cells which are able to change their shape in some degree. The nuclei of these cells are oval and measure about .01 by .008 mm. The vesicle communicates with the exterior by a short narrow muscular tube, around which are numerous nucleated cells. The excretory aperture is at the posterior end of the body.

Genital System.—The genital aperture is situated on a large papilla, which is seen prominently on external inspection. It lies immediately in front of the ventral sucker, and displaced well to the left of the middle line. At first sight the genital sinus appears to be of great size, but this is due to a wide expansion of the ductus ejaculatorius. In reality the genital sinus is comparatively small.

Male Organs.—The testes are two oval bodies symmetrically placed about midway between the ventral sucker and the posterior end of the body. Their long axes are oblique, the posterior end being nearer the middle line; they are separated by the breadth of the excretory vesicle. They are distinctly ventral in position, lying under the ends of the intestinal diverticula. Their average dimensions are .45 by .19 mm., and the thickness equals the breadth. The vas deferens arises from a little nodule about the middle of their inner surface.

The vasa deferentia unite in a small bipartite vesicula seminalis, both parts of which are enclosed within the cirrus-pouch. Jacoby failed to note this bipartite condition, but

there is no doubt it invariably occurs. The posterior part is the smaller of the two. Each has a length of about .085 mm.; the breadth, which is capable of great variation, is respectively .06-.11 mm. and .04-.08 mm. The walls are muscular, and an epithelial lining with a few large nuclei can be observed. The pars prostatica is exceedingly well developed, and, in fact, occupies the largest portion of the cirrus-pouch. It has, as represented in Jacoby's figure, a bulbous shape, narrowed somewhat at its junction with the vesicula seminalis. Its length is .18 mm. and greatest breadth .13 mm. Its wall is muscular and it is lined by a distinct epithelium with oval nuclei, measuring .0096 by .0057 mm. The lumen is almost entirely filled by long string-like masses of prostatic secretion, which lie, like so many filaments, parallel to each other, and are directed out towards the ductus, into which they project. The prostatic cells occupy a large part of the cirrus-pouch, surrounding the pars prostatica and the vesicula seminalis. A few are also found around the ductus. Their nuclei measure .008-.01 mm. Amidst the prostatic cells several large myoblasts or ganglion cells occur. They are distinguished by their much larger nuclei (.012 mm.) and different staining reaction. The pars prostatica passes into a wide (.11 mm.) ductus ejaculatorius, of comparatively short length. Its wall has an irregular outline, being crumpled up in somewhat the same manner as in *Brachycladium oblongum*, but not nearly to the same extent or so regularly. At first sight it might be mistaken for the sinus genitalis, and it certainly offers a contrast to the long narrow ductus found in the majority of Distomids. It is doubtful if it functions as an eversible cirrus, but if so it evidently cannot be everted to any great length. It was not everted in any of my specimens, and Jacoby makes no mention of having seen it in such a condition. The extreme prominence of the genital papilla may be an adaptation to compensate for the shortness of the cirrus. The walls of the ductus have the usual structure, but are unusually thick. The lining consists of a thick metamorphosed epithelium easily distinguished from the cuticular

lining of the sinus genitalis. A small number of "Begleit-zellen" surround the ductus.

Female Organs.—The ovary is situated on the right side of the body immediately in front of the testis, and just behind the ventral sucker. Its shape is not "kegelförmig," as Jacoby describes it, but multilobate, the lobes having apparently no uniform arrangement. This confirms Stafford's observation. It is elongated in a direction parallel to the long axis of the testis, alongside which it lies, and it appears to be a little less than the testis in size. The oviduct arises from its anterior surface and passes behind the ventral sucker towards the dorsal surface. It dilates somewhat before giving off Laurer's canal, and then bends forwards to receive the yolk-duct and pass into the ootype. Laurer's canal is a tube of about .02 mm. diameter, which runs with several small convolutions to open on the middle line of the dorsal surface on the level of the posterior border of the ventral sucker, or a little in front of that. It is surrounded throughout its whole course by numerous small cells. There is no true receptaculum seminis.

The yolk-glands are very limited in extent. They are exclusively lateral, lying to the outer side of, or even slightly ventral to, the ventral sucker. They extend from a little in front of the ventral sucker to near its posterior border. The follicles are few and of small size, and the cells have a great affinity for hæmatoxylin stains. The yolk-ducts run back alongside the intestinal diverticula, and unite in a small median receptacle situated at the level of the anterior end of the testes.

Around the ootype and oviduct there is a fairly compact shell-gland of small size, but with numerous cells, the bodies of which do not stain readily, but the nuclei are very prominent. The latter are oval and about .005 mm. in size. The ootype passes forwards into the uterus, which describes numerous convolutions dorsal to the ventral sucker. The vagina runs straight along the anterior surface of the ventral sucker and opens into the genital sinus. The initial part of

the uterus (about a third of its length) is filled with numerous sperms, and functions as a receptaculum seminis uterinum. The ova are moderate in number and of small size. They are narrow oval in shape, with thick, tough shell, and measure $\cdot 0424$ by $\cdot 0231$ mm.

Fellodistomum agnotum n. sp., Pl. 10, fig. 15.

Among the Distomids which were obtained from the gall-bladder or the adjoining part of the duodenum of *Anarrhichas lupus*, there occurred a few which resembled *Fellodistomum fellis* so closely that they were included along with it. On later and more careful examination, however, they showed features which rendered their specific distinction comparatively easy. My attention was first directed towards these specimens in the course of investigating the size at which *Fellodistomum fellis* attained maturity. It was rather disconcerting to find that some small specimens had numerous ova, while other larger specimens were quite immature. As events have proved, the smaller specimens belong to a distinct species.

Under these circumstances it is difficult to say what the exact habitat of this new species is. As far as my recollection goes, however, these were the specimens which occurred in the duodenum, and the true *Fellodistomum fellis* is apparently confined to the gall-bladder. Unfortunately no further opportunity has offered of confirming this. The new species displays the same green-coloured intestinal diverticula so characteristic of *Fellodistomum fellis*, but this might easily be due to bile ingested in the duodenum.

The chief diagnostic features of the new species are the more elongated body, smaller and less prominent ventral sucker, the forward position of the yolk-glands, and the backward prolongation of the uterus. It is much less numerous than *F. fellis* in the proportion of 1 to 30.

The largest specimen had a length of 3.3 mm. and a

breadth of .87 mm. Other specimens were not quite so attenuated, but the breadth is rarely more than one third of the length. Immature specimens were found up to 1.05 mm. in length. The body usually tapers considerably towards each end. The oral sucker measures .34 mm. and the ventral sucker .51 mm. in a specimen 3 mm. long. The proportion is constantly 2:3 instead of 1:2 as in *F. fellis*. Both suckers are globular. The ventral sucker lies a little in front of the middle of the body.

The alimentary system resembles that of *F. fellis*. The pharynx is somewhat smaller—.15 by .12 mm. The œsophagus is practically absent. The diverticula extend along the sides of the body and terminate at the posterior border of the testes, therefore at a considerable distance from the posterior end of the body. This feature is evidently shared by the genus in common with the genus *Steringophorus*, although it is not apparent at first sight in the case of *Fello-distomum fellis*, in which the testes are placed very near the posterior end of the body.

The excretory system corresponds with that of *F. fellis*, but the main stem of the vesicle is much elongated.

The genital aperture is placed on a prominent papilla in the same situation as in *F. fellis*, and the cirrus-pouch has the same structure. The testes are situated not far behind the posterior border of the ventral sucker, and at a considerable distance from the posterior end of the body. They have the same shape and disposition as in *F. fellis*. The ovary has also the same situation. The yolk-glands, however, lie entirely in front of the ventral sucker and extend along the sides of the body from the anterior border of the sucker to the level of the pharynx or intestinal bifurcation. They form a broader and more compact group than do those in *F. fellis*. The uterus describes a few windings in front of the testes, then runs back between them very nearly to the posterior end of the body. In this part it forms a single descending and ascending loop with little or no convolution. On again reaching the level of the ovary it makes

several irregular convolutions dorsal to the ventral sucker and thence proceeds to the genital aperture. This backward prolongation of the uterus forms a ready means of distinguishing the species with the naked eye from *F. fellis*. The ova are somewhat larger than those of the latter species, measuring about $\cdot 048$ by $\cdot 024$ mm.

For the genus *Fellodistomum*, which Stafford has somewhat scantily characterised, the following definition may be offered :

Small to middle-sized forms with thick fleshy body, sub-cylindrical, tapering more or less towards each end. Cuticle thick and rough, but without spines. Oral sucker sub-terminal, simple. Ventral sucker about the middle of the body, larger than oral sucker, globular. Pharynx small, pre-pharynx short, œsophagus very short or absent. Diverticula, simple wide sacs terminating a little behind the testes. Excretory vesicle Y-shaped, the bifurcation taking place behind the ventral sucker and the limbs stretching far into the neck. Genital aperture situated on a prominent papilla to the left of the middle line immediately in front of the ventral sucker. Small genital sinus. Testes symmetrical, a moderate distance behind the ventral sucker. Cirrus-pouch compact, bulbous. Vesicula seminalis small, bipartite. Pars prostatica well marked ; ductus ejaculatorius short, wide, with walls thrown into irregular folds (in the retracted state). Ovary multilobate, situated just in front of the right testis. Receptaculum seminis absent ; Laurer's canal present. Yolk-glands lateral, of very limited extent. Initial part of uterus functions as receptaculum seminis uterinum. Uterus restricted in extent, either confined between the testes and the genital aperture, or with a loop passing backwards between testes to the posterior end of the body. Ova small, thick-shelled, measuring $\cdot 04$ – $\cdot 05$ mm. by $\cdot 02$ – $\cdot 025$ mm.

Type.—*Fellodistomum fellis* (Olsson, 1868) = ? *F. incisum* (R.) Stafford. Including also *F. agnotum* n. sp.

Both Stafford and Odhner have noted a close resemblance between this genus and the genus *Steringophorus* Odhn.,

1904 (= *Leioderma* Stafford, 1904), of which the type is *St. furciger* (Olss., 1868). The latter genus has been efficiently characterised by Odhner.¹ The two genera show unmistakable evidences of relationship, and they represent a type of structure distinct from that of any other sub-family of the PROSOSTOMATA. They may therefore be regarded as the nucleus of a separate sub-family as already indicated by Odhner, and for this I propose the name *FELLODISTOMINÆ* n. subfam., with the following provisional diagnosis:

Under middle-sized to middle-sized forms with fleshy body. Cuticle unarmed. Ventral sucker larger than oral sucker, situated about the middle of the body. Alimentary canal with short or nearly absent œsophagus, and diverticula not extending much beyond the level of the testes. Excretory vesicle Y-shaped, the fork taking place behind the ventral sucker and the limbs extending well into the neck. Genital aperture a short distance in front of the ventral sucker, median or to the left side. Testes symmetrical, lateral, not far behind the ventral sucker. Cirrus-pouch compact, bulbous, containing a small bipartite vesicula seminalis, a well-marked pars prostatica, and a short, wide ductus ejaculatorius. Ovary in front of right testis, multilobate. Receptaculum seminis absent (or present sometimes), Laurer's canal present. Yolk-glands limited in extent, lateral, on each side of the ventral sucker. Yolk-reservoir median behind ventral sucker. Uterus more or less convoluted, confined between testes and genital aperture or extending back between testes into the posterior part of the body. Ova fairly numerous, measuring about .04–.06 mm. by .02–.03 mm.

Type, *Fellodistomum* (Stafford, 1904).

Including also *Steringophorus*, Odhner.

Genus, *Steringophorus* Odhner, 1904.

Steringophorus cluthensis n. sp., Pl. 10, fig. 16.

This species was found fairly abundantly in the upper

¹ "Trematoden d. arktischen Gebietes," in 'Fauna Arctica,' iv (1904), p. 309.

reaches of the intestine (duodenum and cæca) of *Pleuronectes microcephalus*, the lemon-dab, from the Firth of Clyde. It differs in several respects from *St. furciger* (Olss.), which is found so commonly in many Pleuronectid fishes in the North Sea.

In life the animal is capable of great extension and contraction, but on being killed or on being allowed to die in its natural habitat it assumes a fairly regularly oval outline, always more pointed towards the anterior extremity. It is considerably flattened and has a rather delicate, transparent appearance. Living specimens have a distinctly reddish colour, not so deep as that of *St. furciger*.

The length is 1·5–2 mm., but none of my specimens seem to be fully mature, so they may attain a larger size. The breadth is about two fifths of the length—·6–·8 mm. Cuticle unarmed. The oral sucker invariably lies a short distance from the extreme anterior end, and it is usually elongated in the long axis of the body. It measures about ·22 by ·15 mm. The ventral sucker is almost exactly twice as large, its diameter being ·44 mm. in a specimen of 2 mm. length. It is situated a little behind the middle of the body, and in this respect it differs from the position in *St. furciger*, in which it is in front of the middle of the body.

The pharynx is immediately behind the oral sucker and measures ·085 mm. in diameter. The œsophagus is twice as long as the pharynx, sometimes slightly more, sometimes a little less than that. This feature, again, distinguishes the species from *St. furciger*, in which the œsophagus is about the same length as the pharynx. The diverticula are simple and extend a little beyond the testes, but not so much as in *St. furciger*.

The excretory vesicle is perhaps the most obvious diagnostic feature. It is Y-shaped, but the unpaired portion is very short, so that it sometimes appears almost V-shaped. The paired limbs extend forward to the level of the pharynx. It is distinctly mapped out by the refringent nature of its contents, but is not so conspicuous as in *St. furciger*.

The genital aperture is situated just behind the intestinal bifurcation, to the left of the middle line, although not much. The testes are symmetrically situated midway between the ventral sucker and the end of the body. They are somewhat further back than in *St. furciger*. Their long axes are always a trifle oblique, the anterior end being directed outwards. They measure $\cdot 15$ by $\cdot 12$ mm. The cirrus-pouch is bulbous or nearly globular, and lies entirely in front of the ventral sucker. Its internal structure is the same as that of *St. furciger*, but the pars prostatica is longer and narrower, as are also the two parts of the vesicula seminalis.

The ovary is situated immediately in front of the right testis. It is multilobate, but very small. The yolk-glands are much more extensive than in *St. furciger*. They extend from the testes forward to the level of the middle of the cirrus-pouch, i. e. well in front of the ventral sucker. In *St. furciger* they do not reach the anterior border of the sucker. The transverse yolk-ducts run obliquely backwards to unite at the level of the anterior border of the testes in a small median yolk-reservoir. In none of my specimens was the uterus very voluminous. A few convolutions were found between the testes and stretching back to the posterior end of the body. The terminal part runs forwards on the left side of the ventral sucker to open into the sinus genitalis. The ova are not particularly thin shelled, and measure $\cdot 044$ – $\cdot 056$ by $\cdot 028$ – $\cdot 032$ mm.

The chief diagnostic features of this species may be summed up as follows: Post-acetabular region shorter than pre-acetabular region. Oesophagus twice as long as pharynx. Excretory vesicle nearly V-shaped. Yolk-glands extending in front of ventral sucker.

The introduction of this species within the genus *Sterinogophorus* involves only two modifications of Odhner's definition. The words "Saugnäpfe genähert" and "Stamm (der Exkretionsblase) gabelt sich zwischen den Hoden" should be deleted.

A species which bears a striking superficial resemblance to

Steringophorus cluthensis is *Distomum pagelli* v. Ben. ('Mém. Acad. Roy. Belg.,' xxxviii (1871), Pl. IV, fig. 17), from *Sparus centrodontus*. Van Beneden, unfortunately, gives absolutely no description of the species, but from his figure it seems probable that it is really a *Steringophorus* sp. It differs from *Ster. cluthensis* in having the ventral sucker three times as large as the oral sucker, the ovary globular, the yolk-glands extending behind testes, and the genital aperture further from the intestinal bifurcation.

It is evidently a somewhat difficult matter to differentiate *Steringophorus* generically from *Fellodistomum*. The features which require to be emphasised in the former are: (1) the presence of a distinct œsophagus; (2) the absence of a protuberant genital papilla; (3) the situation of the genital aperture near the intestinal bifurcation instead of close in front of the ventral sucker; and (4) the presence of a true receptaculum seminis.¹ These differences are not of great relative importance, and it is not at all impossible that the genera may eventually prove identical, in which case the name *Steringophorus* must be regarded as a synonym of *Fellodistomum*.

Sub-family *PLAGIORCHINÆ* Pratt, 1902.

Genus *Plagiorchis* Lühe, 1899.

Plagiorchis notabilis n. sp., Pl. 10, fig. 17.

To the genus *Plagiorchis* have already been assigned well-nigh a dozen species, one or two of which can be differentiated from each other only with difficulty. They form on the whole a very homogeneous group. To these I have to add a form possessing such well-marked features that there can be no doubt of its specific distinctness.

¹ According to Odhner there is no receptaculum seminis, but Miss Lebour ('Fish Trematodes of the Northumberland Coast,' p. 15) has shown that such a structure may actually exist in *Steringophorus furciger*.

The species in question was first obtained on August 21st, 1907, from the middle part of the intestine of *Anthus obscurus* (rock pipit), which is by far the commonest bird inhabiting the rocks along the shore in this district. The parasite, however, is by no means frequent, for out of eleven pipits shot during August to October only two specimens were obtained—one adult and one immature. A point of interest is that the two birds from which those specimens were obtained contained several other parasites, in particular two species of *Spelotrema* and a number of Cestodes. The other birds contained only a few examples of *Spelotrema claviforme* and an occasional Cestode. Several other rock-frequenting birds, e.g. *Saxicola œnanthe* and *Motacilla flava*, were examined in the hope of finding more specimens of the parasite, and towards the middle of October another single example was found in the intestine of *Motacilla*. This second example did not entirely agree with the first, but at present they may be regarded as one and the same species. To avoid detailed comparison the specimen from *Anthus* will first be described, and the main features of difference in the specimen from *Motacilla* will then be indicated.

The general shape is that common to the genus. The length is 1·6 mm.; the breadth is fairly uniform—·52–·57 mm. Almost the whole surface of the body is covered with minute straight spines which have the peculiarity that they just barely pierce the cuticle. This feature is shared by the other members of the genus, and would appear to be characteristic.

The oral sucker is not quite at the extreme anterior end of the body, but is a short distance from it. Its length is slightly greater than its breadth, and it has an elongated slit-like aperture. This again appears to be characteristic of the genus. In this species the aperture is slightly expanded posteriorly and narrows to a fine point at the anterior end. The sucker measures ·20 by ·18 mm. The ventral sucker is globular, with a circular aperture, and measures only ·16

mm. It is situated at the end of the first third of the body length.

The pre-pharynx is extremely short; the pharynx is broad and measures $\cdot 07$ by $\cdot 09$ mm. There is practically no œsophagus and the simple straight diverticula extend to within a short distance of the posterior end of the body.

The genital aperture lies immediately in front of the ventral sucker, median or very slightly to the left. The cirrus-pouch bends round to the right side of the ventral sucker. It differs in shape from that of most members of the genus, approaching most nearly that of *Plagiorchis* (*Lepoderma*) *ramlianus* (Lss.). It is short and stout and does not extend beyond the posterior border of the ventral sucker. It is somewhat pointed at its proximal end, where it receives the vas deferens. The vesicula seminalis is oval with only the slightest trace of a constriction, and measures $\cdot 14$ by $\cdot 07$ mm. The prostate cells are fairly numerous, surrounding the first half of the ductus ejaculatorius and pars prostatica. In this specimen the cirrus was exerted as a long, narrow, sinuate filament, pointed at the end and measuring $\cdot 25$ mm. in length. The vagina runs up to the genital aperture on the left side of the ventral sucker. The arrangement of the genital glands is the same as that in the other members of the genus. The anterior testis is $\cdot 20$ mm. behind the ventral sucker, and the posterior testis is $\cdot 35$ mm. from the posterior end of the body. Between the testes there is a space of $\cdot 1$ mm. They are about equal in size, measuring $\cdot 25$ by $\cdot 16$ mm. They are elongated in the long axis of the body, and they lie to the inner side of the intestinal diverticula.

The ovary is situated further forward than in any other species of the genus. It lies on the right side with its anterior border contiguous with the end of the cirrus-pouch, or on the level of aperture of the ventral sucker. It also lies to the inner side of the right intestinal diverticulum, but slightly overlaps it. It is longitudinally oval and measures $\cdot 16$ by $\cdot 12$ mm. From its inner side the oviduct arises and runs towards the middle of the body, where it gives off

Laurer's canal. No receptaculum seminis could be made out with certainty.

The yolk-glands are almost entirely confined to the sides of the body and extend from the pharynx to the posterior end. They do not unite in the middle line posteriorly, but a few follicles stretch across the body just behind the pharynx. The intestinal diverticula are overlapped to a slight extent, especially posteriorly. The transverse yolk-ducts pass a little in front of the anterior testes to unite in a small reservoir.

The uterus is confined to the posterior half of the body and does not overlap the intestinal diverticula. Its course is typical of the genus, but it differs in being more voluminous in front of the posterior testis than behind it. It does not overlap the testes. The ova are not very numerous; they measure $\cdot031$ by $\cdot019$ mm. and have a bright yellow shell.

The chief diagnostic features of this species are therefore the short cirrus-pouch and the forward position of the ovary.

The specimen from *Motacilla flava* was smaller and slightly narrower; length 1.4 mm. Oral sucker $\cdot17$ by $\cdot16$ mm.; aperture $\cdot09$ by $\cdot03$ mm.; thickness of wall $\cdot03$ mm. Ventral sucker two fifths of the body length from the anterior end, size $\cdot14$ by $\cdot13$ mm.; aperture circular. Pharynx $\cdot054$ by $\cdot069$ mm. Spines $\cdot0096$ mm. long.

Cirrus not completely exerted. Vesicula seminalis distinctly bipartite, with a small anterior and a larger posterior part; combined length of the two parts $\cdot066$ mm. The distinct bipartite condition of the vesicula in this specimen is one reason for doubting if it is really identical with the specimen from *Anthus obscurus*. Possibly the difference in the state of exertion of the cirrus may account for the variation in the vesicula.

The genital glands have much the same situation as before, but the posterior testis is only $\cdot12$ mm. from the end of the body and the anterior testis $\cdot13$ mm. behind the ventral sucker. They are smaller and not so elongated, $\cdot17$ by

·15 mm. and ·19 by ·15 mm. respectively. The ovary is also smaller—·115 by ·105 mm. The uterus is more voluminous behind the posterior testis and overlaps the testes to a slight extent. The ova are brownish-yellow and measure ·0308 by ·0193–·0212 mm. The length is practically invariable, but one abnormally large ovum was observed in the posterior loop of the uterus, measuring ·0327 by ·0212 mm. The average size may be taken as ·031 by ·021 mm. They are not truly ellipsoidal, there being a slight flattening on one side, which probably accounts for the variation in the breadth. The shell has a thickness of ·0008 mm. The vagina lies on the right side of the ventral sucker and extends as far back as its posterior border. It is marked off from the uterus by a distinct constriction. Its diameter is ·015 mm. and its walls are ·006 mm. thick.

The yolk-glands are less voluminous than in the first specimen. They are entirely lateral and extend only a short distance in front of the ventral sucker. This might be ascribed to imperfect development, but as both specimens were fully mature it seems more reasonable to allow that some variation may occur in the extent of the yolk-glands. In this respect Braun,¹ in discussing the distinction between *Pl. elegans* (R.) and *Pl. cirratus* (R.), remarks that much weight cannot be placed on the relative extent of the yolk-glands, for they vary even in examples from the same host.

This specimen differs from the first mainly in the condition of the vesicula seminalis, the extent of the yolk-glands, and the size of the genital glands. Until more material is available it is impossible to say whether these features are of specific importance or merely variations.

The species which follow are introduced mainly for the purpose of presenting figures which were omitted from a previous paper.² Little opportunity has offered of making

¹ "Fascioliden der Vögel.," in 'Zool. Jahrb.,' syst. xvi, pp. 37–55, pl. iii, figs. 25–34a.

² "Observations on the Trematode Parasites of British Birds," in 'Annals and Mag. Nat. Hist.' (7), xx (1907), pp. 245–271

fresh investigations in connection with these species, and the notes which are herewith given are only such as to correct a few obvious errors.

Sub-family, MICROPHALLINÆ Ward, 1901.

Genus, *Spelotrema* Jägersk., 1900.

This genus includes the species *Sp. pygmæum* (Levin.), *Sp. claviforme* (Brandes) Mihi., *Sp. simile* Jägersk., and *Sp. excellens* Mihi. A fifth species, namely, *Sp. feriatum* Mihi, has been found to belong to the nearly allied genus *Levinseniella*. The genus may require to be further restricted to three species, for *Sp. claviforme* (Brandes) Mihi is only doubtfully distinct from *Sp. pygmæum*. The characters of the four species may be briefly summarised as follows:

Spelotrema pygmæum (Levins.) Odhn.

Odhn., "Trematoden des arktischen Gebietes" in 'Fauna Arctica,' iv (1904), pp. 315-317, fig. 1, 2A.

Length .3-.5 mm. Maximum breadth .2-.3 mm. Club-shaped. Diameter of suckers about .04-.05 mm., but oral sucker always slightly larger than ventral sucker. Ventral sucker a third of the body length from the posterior end. Intestinal diverticula reaching posterior border of ventral sucker. Genital body only half the breadth of the ventral sucker. Ductus ejaculatorius short and direct. Testes comparatively large. Uterus fairly voluminous, but not obscuring testes. Ova .021-.023 by .012 mm. Habitat, *Somateria mollissima*, *Somateria spectabilis*, *Oidemia nigra*, and *Oidemia fusca*.

Spelotrema claviforme (Brandes) Mihi (Pl. 10, fig. 18).

Nicoll, "Trematode Parasites of British Birds" in 'Annals and Mag. Nat. Hist.,' (7) xx, (1907), pp. 254-255.

Length .2-.4 mm. Maximum breadth .15-.20 mm. Club-

shaped. Oral sucker always distinctly larger than ventral sucker; diameters $\cdot 035$ – $\cdot 04$ mm. and $\cdot 03$ – $\cdot 035$ mm. respectively. Ventral sucker less than a third of the body-length from the posterior end. Intestinal diverticula short, wide apart, and do not reach the level of the ventral sucker. Genital body less than half the diameter of the ventral sucker. Ductus ejaculatorius short and direct. Testes not prominent. Uterus voluminous, obscuring the testes and extending slightly in front of ventral sucker. Ova $\cdot 020$ – $\cdot 024$ by $\cdot 011$ – $\cdot 014$ mm. Habitat, *Pelidna alpina*, *Ægialitis hiaticula*, *Anthus obscurus*,¹ *Numenius arquata*,¹ *Motacilla flava*,¹ *Larus ridibundus*.¹

Spelotrema simile Jägersk.

Jägerskiöld, "Levinsenia pygmæa Levinsen, etc.," in 'Centralbl. f. Bakter.,' Abth. i, Bd. xxvii (1900), pp. 732–740.

Length $\cdot 4$ – $\cdot 6$ mm. Maximum breadth $\cdot 2$ mm. "Biscuit-shaped." Oral sucker slightly smaller than ventral sucker, diameter $\cdot 05$ – $\cdot 06$ mm. Intestinal diverticula reach posterior border of ventral sucker. Genital body has diameter at least two thirds that of the ventral sucker. Ductus ejaculatorius long and convoluted. Uterus not usually voluminous, not obscuring testes or yolk-glands. Ova pale, measuring $\cdot 023$ – $\cdot 026$ by $\cdot 011$ – $\cdot 013$ mm. Habitat, *Larus argentatus*, *Larus fuscus*, *Larus ridibundus*.¹

Spelotrema excellens, Mihi (Pl. 10, fig. 19).

Nicoll, 'Annals and Mag. Nat. Hist.,' (7) xx, (1907), pp. 248–251.

Length $\cdot 7$ – $1\cdot 4$ mm. Maximum breadth $\cdot 35$ – $\cdot 50$ mm. Club-shaped. Oral sucker slightly larger than ventral sucker; diameter $\cdot 06$ – $\cdot 085$ mm. Intestinal diverticula terminate at the level of the centre of the ventral sucker. Genital body nearly as large as ventral sucker. Ductus ejaculatorius short and straight. Uterus very voluminous, obscuring testes, but not extending in front of ventral sucker as a rule. Ova

¹ New hosts

very numerous, measuring $\cdot 023$ – $\cdot 025$ by $\cdot 010$ – $\cdot 013$ mm. Habitat, *Larus argentatus*.

Genus, *Levinseniella* Stiles, 1902.

The species of this genus, namely *L. brachysoma* (Crepl.), *L. pellucida* Jägersk., and *L. propinqua* Jägersk., have been efficiently described by Jägerskiöld.¹ The species which I have described under the name *Spelotrema feriatum* n. sp.² must be considered as a mixture of two or more of the above mentioned species or of some hitherto undescribed species of the same genus. I found the species originally in *Hæmatopus ostralegus*, and was struck with its resemblance to *Distomum brachysomum* Crepl., but was led astray by Villot's transposed representation³ of Creplin's species. Jägerskiöld's elucidation of the true structure of that species has enabled me to identify my specimens as *Levinseniella brachysoma* or some nearly related species.

At the time of describing the species "*Spelotrema feriatum*," I entertained great doubt as to the actual identity of my specimens from *Vanellus* and *Hæmatopus* with those from *Pelidna* and *Ægialitis*. This doubt has now given place almost to a certainty that they are distinctly different, for the former, although quite as large as the latter, were in every case decidedly immature and pale in colour, while the latter contained numerous ova and were of a more or less brownish colour. According to Jägerskiöld, *Hæmatopus* harbours a species distinct from that inhabiting *Ægialitis*, *Totanus* and *Pelidna*. This would appear to solve the difficulty with regard to my specimens, but unfortunately I cannot entirely reconcile my own observations with Jägerskiöld's descriptions. For instance, the brown pigmentation of the body and the intensely black mapping

¹ "Zur Kenntniss der Trematodengattung *Levinseniella*," in 'Zool. Studien tillägnade,' Prof. T. Tullberg, 1907, pp. 133–154.

² 'Annals and Mag. Nat. Hist.,' (7), xx, pp. 251–253.

³ 'Ann. d. Sciences Nat.,' (6), viii (1879), pp. 22–24, pl. v, fig. 7.

out of the excretory system are not mentioned by Jägerskiöld. I have endeavoured to utilise the table of specific differences given by Jägerskiöld¹, but without any consistent result. From the point of view of the comparative length of the pre-pharynx, for instance, my specimens from *Totanus* and *Vanellus* ought to be regarded as *L. propinqua* but the identity is not confirmed in other respects. For the present it seems the safest plan to refer my specimens from *Pelidna*, *Ægialitis* and *Totanus* to *Levinsinella brachysoma* (Crepl.), while those from *Hæmatopus* and *Vanellus* must be regarded as *Levinsinella* sp. *inquir.* The most curious feature about the latter is the fact that on no occasion did I find a fully mature specimen, although I examined several of these birds at different times. In addition to the foregoing hosts I have also to add *Numenius arquata*, in the cæca of which I have found a *Levinsinella* sp., probably *L. brachysoma*.

Sub-family *Tocotreminæ* Jägerskiöld, 1902.

Genus *Tocotrema* Looss, 1899.

Tocotrema jejunum Mihi (Pl. 10, figs. 20 and 21).

I have not yet again met with this species. Two figures of it are shown here, one representing what may be regarded as the normal shape, the other that in which it was usually found.

Genus *Cryptocotyle* (Lühe) Mihi, 1907.

Cryptocotyle concava (Crepl.) (Pl. 10, figs. 24 and 25).

My revised definition of this genus requires alteration in, at least, one point. From a rather poor series of sections I have been able to make out that the genital sucker contains a distinct plug-shaped body ("kegelförmiger Körper"). It is, however, much smaller than the corresponding structure

¹ Op. cit., p. 147.

in *Tocotrema*. This fact removes an important point of distinction between the genera *Cryptocotyle* and *Tocotrema*, but sufficient differences remain to keep them generically separate.

In my previous note on Looss's sub-family *Cœnogoniminæ* (*Heterophyinae*), I arrived at practically the same conclusion as Jägerskiöld did in a paper¹ which I had not at that time seen. Jägerskiöld separates the sub-family *Tocotremiæ* from the *Cœnogoniminæ*, including in the former the same genera, with the exception of *Cryptocotyle*, as I did later. He also ventures the supposition that the *Microphallinae* are more or less nearly related to the *Tocotremiæ*-*Cœnogoniminæ* group, and this seems not at all unreasonable. Their relation to the *Brachycœliinae* is undoubtedly much more remote, and my suggestion of a natural family to include all these forms was certainly premature if not erroneous.

Sub-family *Gymnophallinae* Odhn., 1904.

Genus *Gymnophallus* Odhn., 1900.

Gymnophallus dapsilis Mihi (Pl. 10, fig. 24).

An explanation of the curious condition of the ova in this species is suggested in a note by Looss,² who has met with an analogous condition in several other species, and ascribes it to an imperfect functioning of the ootype. The malformed ova may therefore in a sense be regarded as abortive. I have since noted the condition in one or two other species, but only in isolated individuals, and never with the same remarkable frequency as in *Gymnophallus dapsilis*.

Sub-family *Maritreminæ* provis.

Genus *Maritrema* Mihi, 1907.

I have at present nothing further to add to the descriptions

¹ "*Scaphanocephalus expansus* (Crepl.), etc.," in 'Results of Swedish Zool. Exped. to Egypt,' 1901, No. 23.

² "*Trematoden sus Seeschildkröten*," in 'Zool. Jahrb. Syst.,' xvi, p. 475.

of the three species of this genus. They are represented here in Pl. 10, figs. 25-27.

Dr. Jägerskiöld has just sent me a paper dealing with the genera *Spelotrema* and *Maritrema* (in 'Centralbl. f. Bakt., etc., I Abt. Originale,' Bd. xlviii, pp. 302-317). His description of *Spelotrema excellens* appears to agree very closely with mine. The elongated shape of the ovary is certainly not a constant feature of the species, nor is the large size of the vesicula seminalis, on which Jägerskiöld lays some weight. It is an organ which is capable of not a little variation in size at different times in the same animal.

With regard to the genus *Maritrema*, Jägerskiöld's two new species appear to be quite distinct from those which I have previously described. He is correct in drawing attention to the disposition of the yolk-glands. The genus, as Jägerskiöld points out, shows a relationship to the *MICROPHALLINÆ*, but it is certainly not close enough to permit of its being included in that sub-family.

EXPLANATION OF PLATES 9 AND 10.

Illustrating Dr. William Nicoll's paper entitled "Studies on the Structure and Classification of the Digenetic Trematodes."

The following letters apply to all the figures:

B.S. Ventral sucker. *C.B.* Cirrus-pouch. *D.E.* Ductus ejaculatorius. *D.St.* Yolk-glands. *Ex.* Excretory vesicle. *J.* Intestinal diverticula. *K.St.* Ovary. *L.C.* Laurer's canal. *M.S.* Oral sucker. *Oe.* Œsophagus. *Ov.* Ova. *P.G.* Genital aperture. *Ph.* Pharynx. *P.Ph.* Pre-pharynx. *P.Pr.* Pars prostatica. *Pr.* Prostate glands. *T₁, T₂.* Testes. *R.S.* Receptaculum seminis. *S.D.* Shell-gland. *Ut.* Uterus. *Vg.* Vagina. *V.S.* Vesicula seminalis.

FIG. 1.—*Stephanophiala laureata* (Zed.). Ventral view. $\times 35$. *V.P.* Ventral papilla.

FIG. 2.—*Steph. laureata*. Longitudinal section of anterior end, nearly median. $\times 85$. *V.P.* Ventral papilla. *D.P.* Dorsal papilla. *a.* Myoblast.

FIG. 3.—*Steph. laureata*. Cirrus-pouch and vagina. Somewhat diagrammatic. $\times 270$. σ . Male genital aperture. ϕ . Female aperture.

FIG. 4.—*Steph. laureata*. Transverse section, yolk-follicles. $\times 270$. *P.C.* Parietal cell. *C.C.* Central cell. *D.G.* Yolk-duct. *Y.C.* Yolk-cell.

FIG. 5.—*Steph. laureata*. Immature specimen. $\times 60$. *E.S.* Eye spot.

FIG. 6.—*Brachycladium oblongum* (Brn.). Shell-gland complex. Transverse section. *K.G.* Oviduct. *D.G.* Yolk-duct. *Cu.* Cuticle. *D.R.* Yolk-reservoir. *V.D.* Vas deferens. *R.S.* *Ut.* Receptaculum seminis uterinum. *Oo.* Ootype.

FIG. 7.—*Brachycladium oblongum*. Longitudinal median section through genital aperture. $\times 60$.

FIG. 8.—*Brachycladium oblongum*. Longitudinal median section through anterior end, showing pre-pharyngeal pouch. Reconstructed. $\times 60$. *Div.* Pre-pharyngeal pouch.

FIG. 9.—*Lebouria idonea* n. sp. Ventral view. $\times 50$. *D.R.* Yolk reservoir.

FIG. 10.—*Lebouria idonea*. Coronal section, anterior end. $\times 100$. *I.D.* Intestinal dilatation.

FIG. 11.—*Lebouria idonea*. Cirrus-pouch. $\times 330$. *B.Z.* "Begleit-zellen."

FIG. 12.—*Lebouria idonea*. Shell-gland complex. Dorsal view. $\times 85$. *K.G.* Oviduct. *D.G.* Yolk-duct. *D.R.* Yolk-reservoir.

FIG. 13.—*Fellodistomum fellis* (Olsson). Longitudinal section through dorsal surface and intestinal diverticulum just in front of ventral sucker. $\times 80$. *Cu.* Cuticle with rod-like bodies. *Ep.* Intestinal epithelial cells containing masses of bile (*Z.*).

FIG. 14.—*Fellodistomum fellis*. Transverse section through centre of ventral sucker. $\times 75$. *C.M.* Circular muscle-fibres of sucker. *D.Z.* Subcutaneous gland cells. *D.Z.S.* Gland cells of the sucker. *G.Z.* "Grosse zellen" (myoblasts?). *I.Z.* Cells of the inner zone. *M.Z.* Cells of the median zone. *P.Z.* Cells of the outer (peripheral) zone.

FIG. 15.—*Fellodistomum agnotum* n. sp. Ventral view. $\times 35$.

FIG. 16.—*Steringophorus cluthensis* n. sp. Ventral view. $\times 55$.

FIG. 17.—*Plagiorchis notabilis* n. sp. Ventral view. $\times 55$. *C.* Cirrus.

FIG. 18.—*Spelotrema claviforme* (Brandes). Ventral view. $\times 200$. *G.S.* Genital sucker.

FIG. 19.—*Spelotrema excellens* Mihi. Ventral view. $\times 130$. *G.S.* Genital sucker.

FIG. 20.—*Tocotrema jejunum* Mihi. Ventral view. $\times 65$. *G.S.* Genital sucker.

FIG. 21.—*Tocotrema jejunum*. Ventral view. Greatly extended specimen. $\times 65$. *G.S.* Genital sucker.

FIG. 22.—*Cryptocotyle concava* (Crepl.). Ventral view. $\times 95$. *G.S.* Genital sucker. *D.R.* Yolk reservoir.

FIG. 23.—*Cryptocotyle concava*. Median longitudinal section. $\times 240$. *G.S.* Genital sucker. *Z.* "Zunge." *Ov.* Ovum.

FIG. 24.—*Gymnophallus dapsilis* Mihi. Ventral view. $\times 95$.

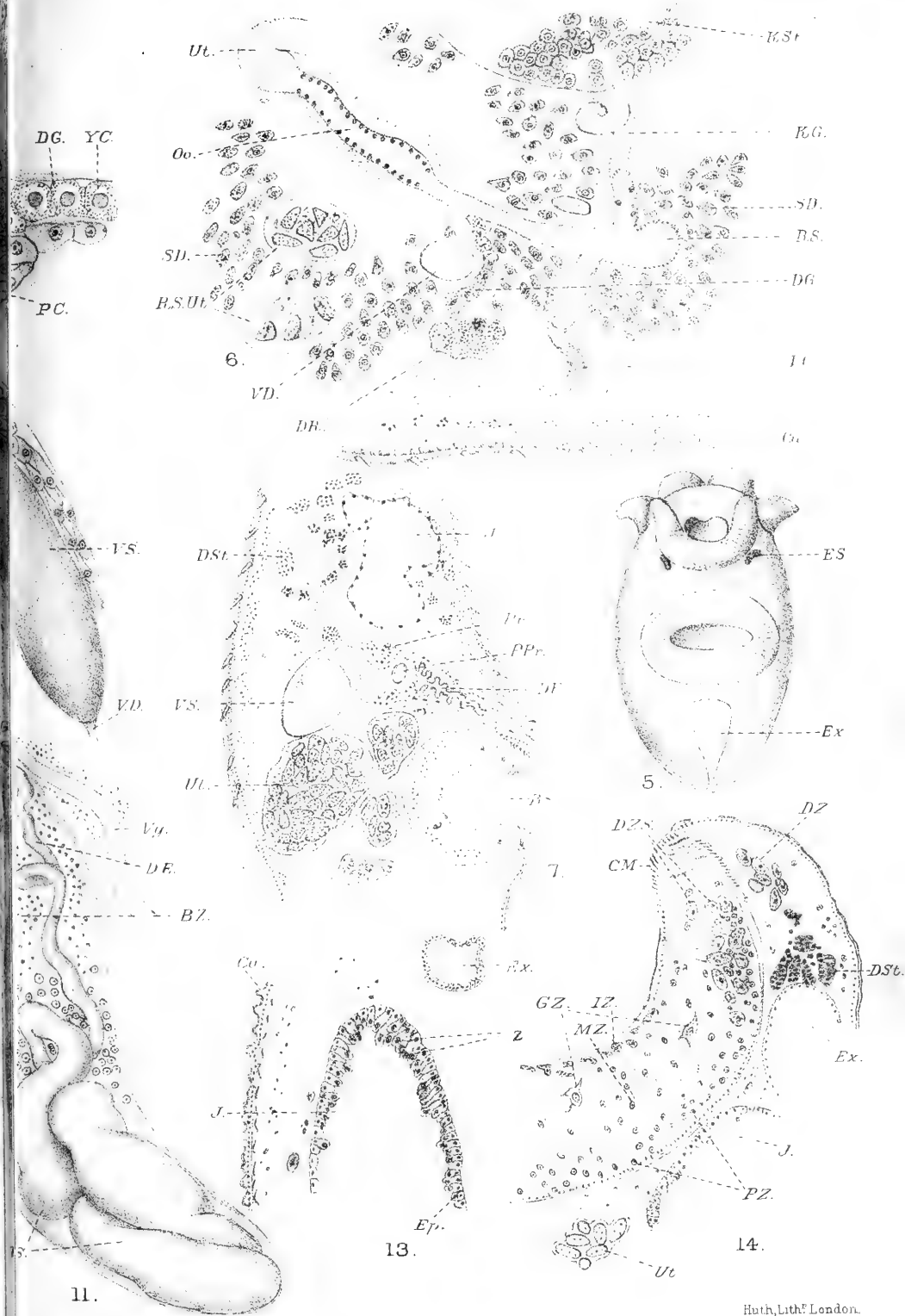
FIG. 25.—*Maritrema gratiosum* Mihi. Ventral view. $\times 110$.

FIG. 26.—*Maritrema lepidum* Mihi. Ventral view. $\times 100$.

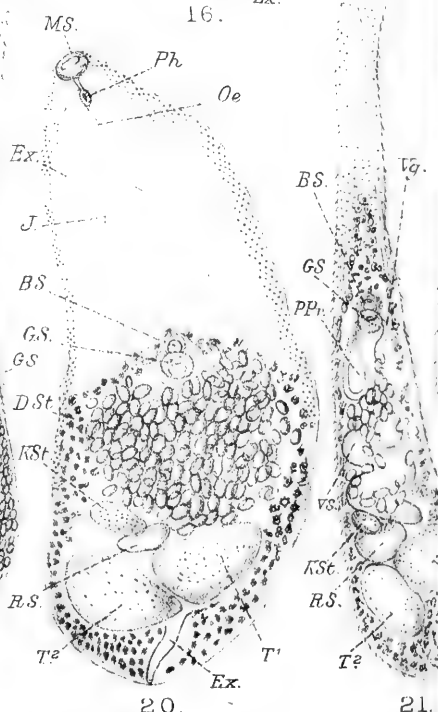
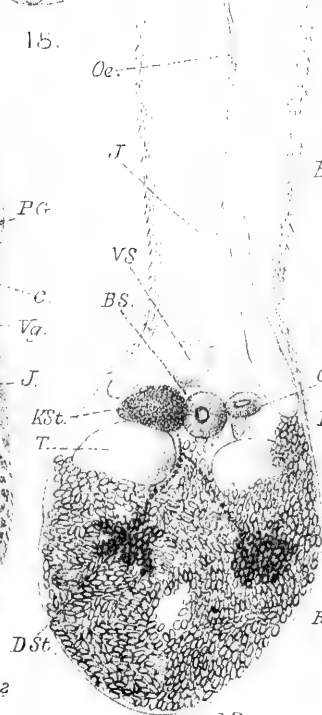
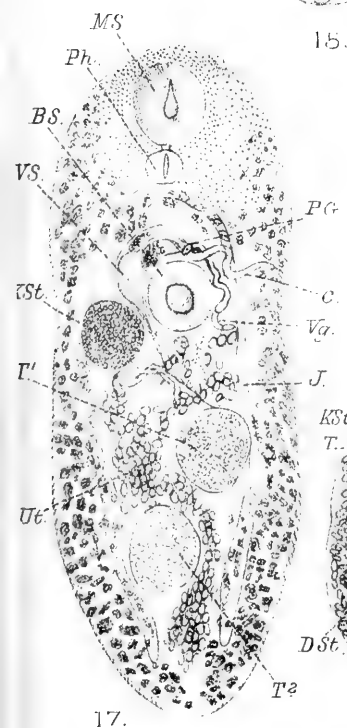
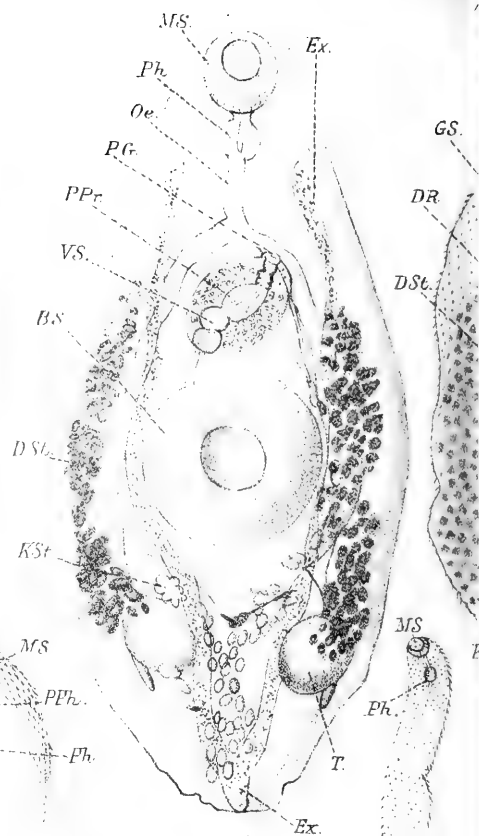
FIG. 27.—*Maritrema humile* Mihi. Ventral view. $\times 180$.

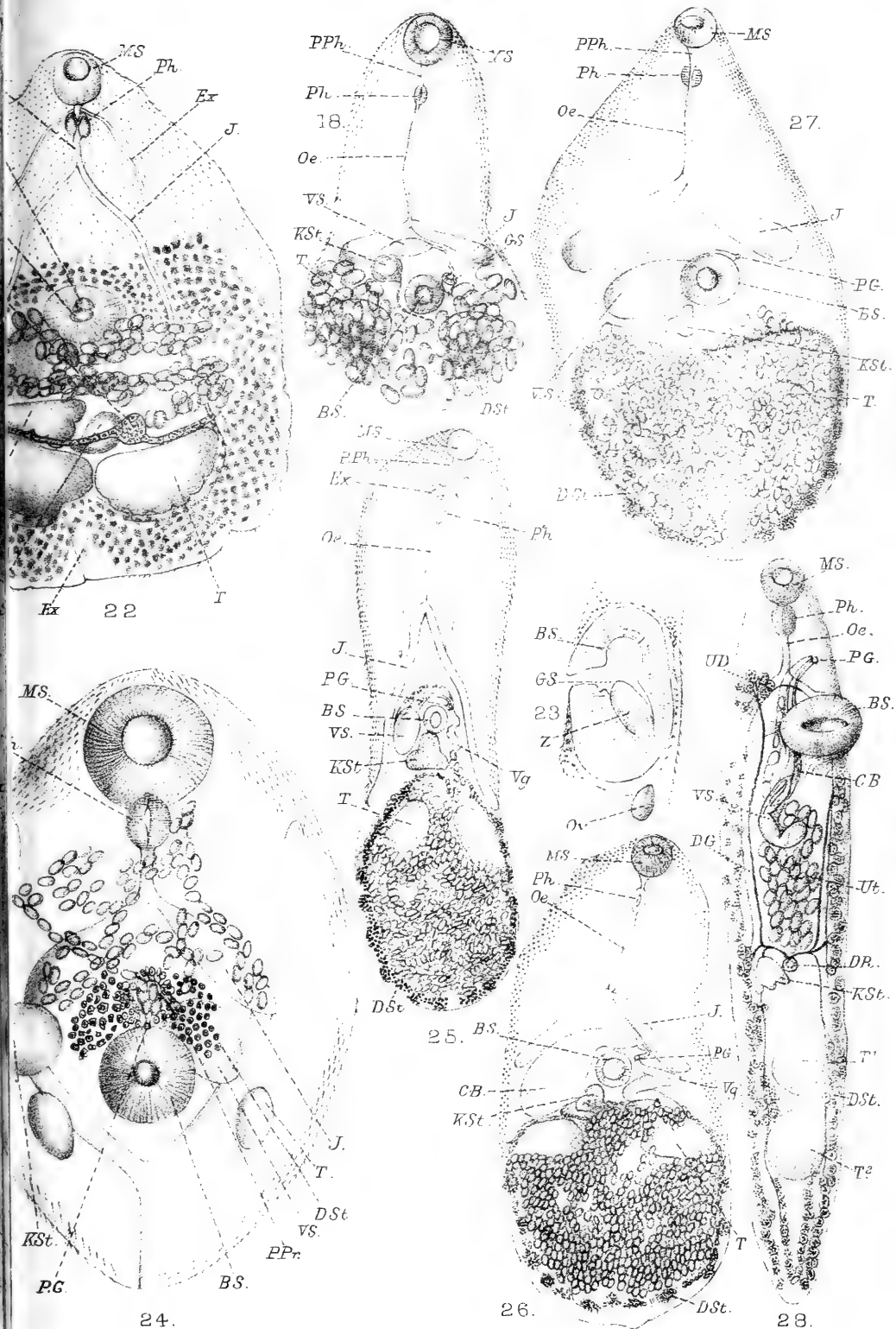
FIG. 28.—*Podocotyle atomon* var. *dispar*. Ventral view. $\times 45$. *U.D.* Unpaired group of yolk-follicles. *D.R.* Yolk reservoir. *D.G.* Longitudinal yolk-duct.













On the Anaspidacea, Living and Fossil.

By

Geoffrey Smith,

Fellow of New College, Oxford.

With Plates 11 & 12 and 62 Text-figures.

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I. HISTORICAL INTRODUCTION.

THE first members of the Anaspidacea to be discovered and described were certain fossil forms occurring in the Permian

and Carboniferous strata of Europe and North America, and it was not until long afterwards that a living representative was found in the fresh waters of Tasmania and recognised as the near relative of these very ancient fossils.

In 1856 Jordan and von Meyer (1) described a fossil shrimp from the Permo-Carboniferous of Saarbrück which they named *Gamponyx fimbriatus*, and which was seen to combine certain features of the Podophthalmate Crustacea with the entire absence of a carapace.

In 1865 and 1868 Meek and Worthen (2 and 3) figured two similar forms from the Coal-measures of Illinois which they named *Acanthotelson stimpsoni* and *Palæocaris typus*.

The systematic position of these fossils remained obscure until Packard re-examined them, and in a series of papers (4, 5 and 6) did a good deal to elucidate their structure and affinities. He instituted the use of the term "Syncarida" to include the three genera and to designate a group of the higher Crustacea intermediate in its characters between the Schizopoda and the Edriophthalmata. Packard's conception of the affinities of this group have been borne out by recent investigations, and his name "Syncarida" has been adopted by Calman as one of the divisions of the Malacostraca.

In 1893 Thomson (7) gave an account of a remarkable freshwater shrimp from the top of Mount Wellington, Tasmania, which was pointed out to him by Mr. Rodway, of Hobart, and which had been known to, and even occasionally eaten by, the settlers for some time. Thomson named the animal *Anaspides tasmaniae*, described the most important points of its external anatomy, and decided that it belonged to the sub-order Schizopoda, of which he considered it the most primitive member.

The connection between *Anaspides* and the fossil *Syncarida* was first pointed out by Calman (8), who revised Thomson's description in certain particulars, and drew a careful comparison between *Anaspides* and the Carboni-

ferous fossils, concluding therefrom that they all belonged to the same order and are very closely allied.

In a subsequent paper (9) the same author amplifies and crystallises the views of Boas and Hansen on the classification of the Malacostraca, and proposes to do away with the order Schizopoda and to redistribute its component families, uniting the Euphausiidae with the Decapoda to form a division Eucarida, while the Eucopiidae, Lophogastridae and Mysidae are united with the Cumacea, Amphipoda and Isopoda as Peracarida. The Anaspididae are placed in a separate subdivision, the Syncarida, together with the three fossil forms mentioned above.

Calman gives the following diagnosis of the three divisions:

Division Eucarida.—Carapace coalescing dorsally with all the thoracic somites. Eyes pedunculate. Antennal protopodite with, at most, two distinct segments. Mandible without lacinia mobilis in adult. Thoracic limbs flexed between fourth and fifth segments. No oostegites. An appendix interna sometimes present on pleopods. Hepatic cæca much ramified. Heart abbreviated, thoracic. Spermatozoa spherical or vesicular, often with radiating appendages. Development, as a rule, with metamorphosis.

Division Peracarida.—Carapace, when present, leaving at least four thoracic segments distinct. First thoracic segment always fused with the head. Antennal protopodite typically of three segments. Mandible with lacinia mobilis (except in parasitic and other modified forms). Thoracic limbs flexed between fifth and sixth segments. Oostegites attached to some or all of the thoracic limbs in female, forming a brood-pouch. No appendix interna on pleopods. Hepatic cæca few and simple. Heart elongated, extending through the greater part of the thoracic region, or displaced into abdomen. Spermatozoa filiform. Development taking place within brood-pouch; young set free at a late stage.

Division Syncarida.—Carapace absent. All the thoracic segments distinct. Eyes pedunculate. Antennal protopodite of two segments. Mandible without lacinia mobilis. Thoracic

limbs flexed between fifth and sixth segments. No oostegites. No appendix interna on pleopods. Hepatic caeca numerous. Heart elongated, tubular.

Largely as the result of Calman's writings, the importance of Anaspides, both as the sole survivor of a group of Crustacea, otherwise known only from the Permo-Carboniferous seas of the northern hemisphere, and as the representative of probably the most primitive Malacostracan division, became obvious, so that it was clearly desirable to learn more about its habits and internal anatomy, and to find out if other allied forms were still existing in the freshwaters of the southern hemisphere.

In the autumn of 1907, at the suggestion and through the assistance of Professor G. C. Bourne, I went to Tasmania to investigate Anaspides. On arriving in Melbourne I met Mr. O. A. Sayce, of Melbourne University, and learnt that a few weeks before my arrival he had obtained some specimens of a freshwater Crustacean, which he believed to be closely related to Anaspides, from a small stream to the west of Melbourne. Mr. Sayce has subsequently published an account of the animal, which he calls *Koonunga cursor* (10 and 11), belonging to a separate family, Koonungidae of the Anaspidacea. Perhaps the most interesting point about this animal is the fact that, unlike Anaspides and all other Schizopods, it possesses sessile eyes, a characteristic which tends to break down the old distinction between Podophthalmata and Edriophthalmata, a distinction which Calman's classification also ignores.

My own investigations in Tasmania were directed chiefly towards the elucidation of the obscure points in the habits and internal anatomy of *Anaspides tasmaniae*, and a preliminary account (12) of these matters was published on my return in June, 1908. I was also able to report the discovery of a new species and genus of the Anaspididae, *Paranaspides lacustris*, from the great Lake of Tasmania. As a result of my studies I inclined to the conclusion that the Anaspidacea, while possessing many peculiar features, were

related by certain characters—e. g. filiform spermatozoa and the structure of the heart—to the Peracarida, and by others to the Decapoda, a conclusion which has been subsequently confirmed. The composite character of the Anaspidacea, which seem to be constructed by uniting characteristics taken from the other divisions of the Malacostraca, appeared to me to point to the extremely primitive nature of the group, and to confirm Calman's opinion that they should be separated from the other Malacostracan divisions as a discrete group, the Syncarida.

In the September number of the 'Geological Magazine' for 1908, Dr. Henry Woodward (13) describes for the first time some specimens of a fossil crustacean from the Coal-measures near Ilkeston, Derbyshire, which must be considered as the most perfectly preserved specimens of fossil Syncarida that have as yet been found. Dr. Woodward names them *Præanaspidēs præcursor*, and there can be no doubt that they represent an exceedingly close ally of the living Anaspidacea. The details of segmentation, of the form of the limbs, and the general posture of the body in *Præanaspidēs* are exactly reproduced in the living *Anaspidēs* or *Koonunga*, and we are amply justified in placing this ancient palæozoic fossil together with the living genera in the same order or even in a nearer relationship (text-fig. 3).

Our knowledge therefore of this interesting group of primitive Crustacea is beginning to take definite shape, and since in the future it must always hold a prominent position in Crustacean morphology and classification, it is, perhaps, timely to bring together all we know about these animals in a systematic form, and to attempt to determine their place in classification, and the light which they throw upon the evolution of the higher Crustacea.

Before leaving the historical aspect of our subject, reference must be made to Professor Fritsch's views upon the affinities of the fossils which he has described (17), from the carboniferous strata of Bohemia. In his admirable memoir he describes a fossil Malacostracan, *Gasocaris*

Krejci, which he places together with *Gampsonyx*, *Palæocaris*, *Acanthotelson* and *Mectotelson* in a sub-order of the Podophthalmata, which he names *Simplipoda*. Professor Fritsch believes that *Gasocaris* had simple uniramous thoracic limbs, and he also ascribes this character to the other genera, despite Packard's assertion in regard to *Palæocaris*, and the apparent condition of *Gampsonyx*. Professor Fritsch denies that any of these forms is related to *Anaspides*, or to any other Schizopod, on the ground of their possessing uniramous limbs. As Calman (18) has pointed out, the mere fact, if it were established, that some of these fossil forms had uniramous limbs would not invalidate the conclusion that they are related to *Anaspides*. This relationship is established more effectively by such common characters as the lack of a carapace, the eight free thoracic segments, the pedunculated eyes and form of the antennæ, tail-fan and telson. With regard to the possession of biramous limbs, we know now that *Præanaspides* exhibited this character, and the same is true of *Palæocaris* and perhaps of *Gampsonyx*. It must also be remembered that the exopodites of the thoracic limbs in *Anaspides* and its living allies are exceedingly slender and delicate structures, and that even in the beautifully preserved fossil *Præanaspides* they are by no means easy to be made out, though they are demonstrably present. We cannot therefore attach great weight to Professor Fritsch's assertion that they are altogether absent in *Gasocaris* and *Gampsonyx*, and even if this is the case, it would not alter our conviction that these forms are closely related to *Anaspides*. The fossil, *Gasocaris* (see text-figs. 59, 60, 61), the details of whose structure Professor Fritsch so beautifully illustrates, reproduces with great exactitude the essential features of *Anaspides*. The pedunculated eyes, the first antennæ with three jointed peduncles, the second antennæ with their scales, the entire absence of a carapace, the form of the telson and tail-fan, are all nearly identical with the corresponding features in *Anaspides*.

With regard to the segmentation of the body, Professor Fritsch confesses that he is doubtful, but the number of segments which he gives in his restored figure is plainly wrong. He only figures six thoracic segments in the restored figure, but it appears to be demonstrable from his figure of an actual specimen in ventral view that there are eight free thoracic segments carrying eight similar limbs. It is impossible not to observe that if only Professor Fritsch, at the time of writing, had been familiar with the living Anaspides, he would have interpreted his fossils otherwise. But what shall we say of the restoration of *Gampsonyx*, in which, according to Professor Fritsch, there were seven abdominal somites besides the telson, and two pairs of maxillipeds in front of the seven pairs of thoracic legs? As Calman points out, these characters are so exceedingly peculiar as to preclude direct comparison with any other known Crustacean, and would remove *Gampsonyx* from any immediate relationship to the Malacostraca at all. While gratefully acknowledging, therefore, Professor Fritsch's careful descriptions of these interesting fossils, we find it impossible to follow him in his general restorations of them, or in his denial of their relationship to the Anaspidacea. There is one other point in Professor Fritsch's work which may excite a comment, and that is the alleged presence of an otocyst on the inner ramus of the uropod in *Gasocaris* and *Gampsonyx*. An otocyst in this position is only found elsewhere among the Mysidacea; it is not present in the living Anaspidacea or in Præanaspides.

2. EXTERNAL MORPHOLOGY.

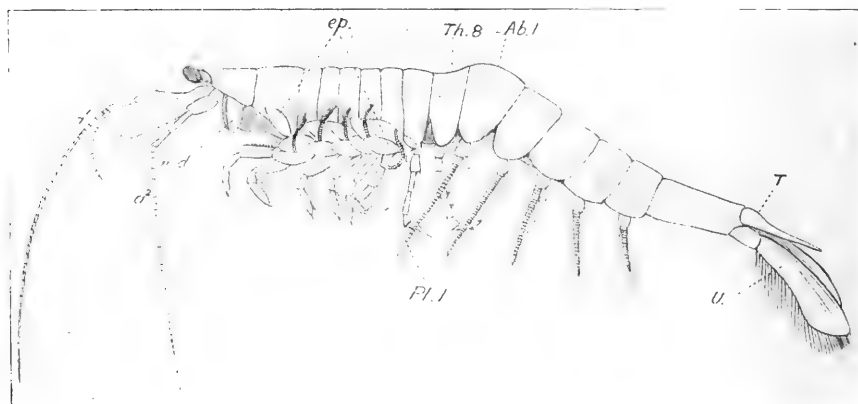
(A) General Appearance.

The Syncarida are rather small animals, the largest size being attained by the living *Anaspides tasmaniae*, exceptional specimens of which may measure over two inches in length. The smallest form known is the living *Koonunga cursor*, which measures about a quarter of an inch in length.

We may describe, as a type, the general appearance of *A. tasmaniae*, the mountain-shrimp of Tasmania, found in the pools of rivers and in tarns at a high elevation. The chitinous integument is soft and uncalcified, and of a straw-yellow colour; beneath it in the skin are numerous branching black chromatophores, arranged in a similar pattern on each segment. Along the dorsal middle line two dark lines are visible, which are caused by the pigmentation on the floor of the pericardium.

In the natural position the body is held straight and un-

TEXT-FIG. 1.



Paranaspides lacustris, ♀. Lateral view. $\times 4$. *a¹*. First antenna. *a²*. Second antenna. *md*. Mandible. *ep*. Gills. *Th. 8*. Eighth thoracic segment. *Ab. 1*. First abdominal segment. *Pl. 1*. First abdominal appendages. *T*. Telson. *U*. Uropod.

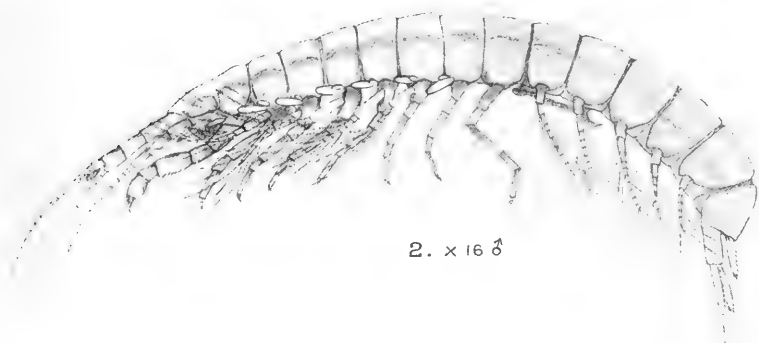
flexed with the limbs disposed in the characteristic manner shown in Pl. 11, fig. 1. The normal habit of the animal is to walk or run upon the stones at the sides or bottom of the deep pools in which it lives; this walking movement is effected by the endopodites of the eight thoracic limbs, but it is also assisted by the long exopodites of the abdominal appendages. The exopodites of the thoracic limbs are kept in a continual waving motion, and no doubt aid in respiration by agitating

the water round the delicate leaf-like external gills attached to the bases of the thoracic limbs.

The body consists of a head, bearing a pair of pedunculated eyes, and there follow apparently eight free thoracic segments, six abdominal segments and a telson. The sixth abdominal segment carries a pair of expanded, backwardly directed pleopods, which form a powerful tail-fan.

Paranaspides lacustris (text-fig. 1, and Pl. 11, fig. 2), from the Great Lake of Tasmania, although in its detailed

TEXT-FIG. 2.



Koonunga cursor, ♂, from a drawing by Mr. Sayce. $\times 16$.

structure very similar to *Anaspides*, differs very widely from it in external appearance, and in this respect it is probably the most aberrant of all the Syncarida, including the fossil forms. The body, instead of being deeply pigmented, is of a transparent green colour, sparsely powdered with black dots; and there is a very marked dorsal flexure. The abdomen is elongated, the tail-fan enlarged, the exopoditic scales of the second antennæ also enlarged, and the eyes are borne on elongated stalks. All these characters, which differentiate *Paranaspides* from the other Anaspidacea, are correlated with the habits of the animal, which

lives among weeds in the littoral region of the lake, rather after the manner of a prawn, and pursues more of a swimming habit than the rest of the order to which it belongs. This habit and the characters correlated with it are therefore most probably a fairly recent acquisition.

The other living representative, *Koonunga cursor*, is a little marbled-grey animal which differs from *Anaspides* in several important characters, such as the possession of sessile in place of stalked eyes, the entire absence of a scale on the second antennæ, and the presence of only seven free thoracic segments, but it closely resembles *Anaspides* in general appearance, especially in its habit of running with the body held straight and unflexed.

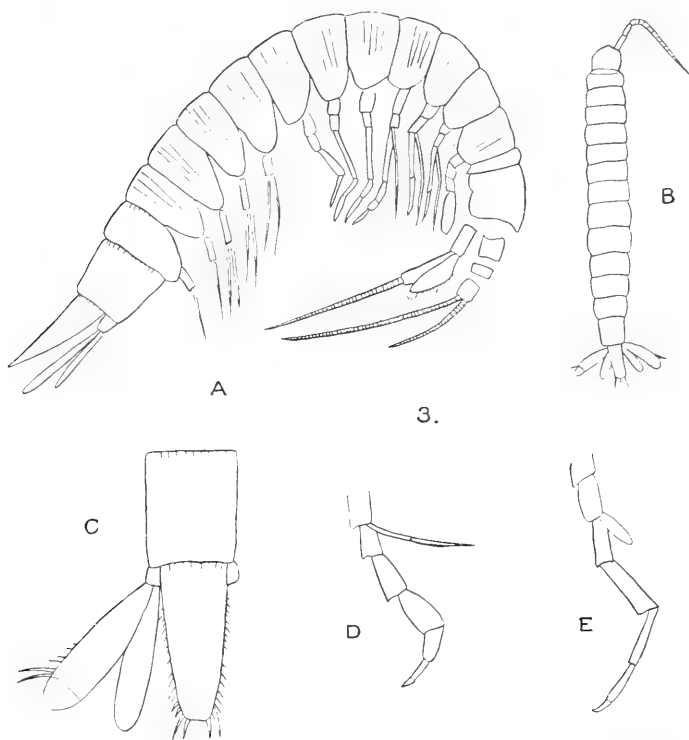
Of the fossil forms we can say for certain that they followed a similar mode of life to *Anaspides* and *Koonunga*. There is no trace in any of them of a true dorsal flexure, the fossils being in many cases preserved with the body quite straight as in the normal walking position of *Anaspides*. The tail-fan is small, the external scales not enlarged, and the eyes either shortly pedunculated or possibly in some cases absent.

The most perfect resemblance to *Anaspides* is afforded by the English carboniferous fossil *Præanaspides*, described by Woodward. The segmentation and posture of the body, the detailed jointing of the limbs and antennæ in this fossil so exactly reproduce the corresponding features of the living *Anaspides*, that so far from there being any doubt as to the two forms being referable to the same order, it is justifiable to include them in the same family. The entire absence of the characteristic leaf-like gills in this and all the other fossil *Syn-carida* is unfortunate, but we could hardly hope that these extremely delicate and perishable structures should be preserved for us in a fossil state.

With regard to the other fossils, although there can be small doubt that we are dealing with allied forms, it is difficult to be certain about details. *Gampsonyx* (text-fig. 53) has a very similar body form and segmentation to *Anaspides*,

and apparently some of the thoracic limbs were biramous. The first thoracic limb was, however, raptorial and greatly enlarged. There is less doubt about *Palæocaris* (text-figs. 56, 57), as the thoracic limbs are distinctly biramous, there

TEXT-FIG. 3.



Præanaspidites præcursor, after Henry Woodward. A. Lateral view. B. Dorsal view. C. Telson and uropods. D. Fourth thoracic limb. E. Seventh thoracic limb.

are eight free thoracic segments, and the antennæ and tail-fan are very similar to those structures in *Anaspides*. The eyes are unfortunately unknown.

Gasocaris (text-fig. 59), despite Professor Fritsch's assertion that the limbs are uniramous, was certainly a typical member of the Anaspidacea in all other respects.

Acanthotelson (text-fig. 62) appears to me to occupy a different position, and I am doubtful if it is rightly associated with the *Syncarida* at all. The thorax only possessed seven free segments, and the thoracic limbs show no trace of being biramous. The abdominal limbs are expanded, flabellate structures, and the tail-fan is elongated and sharply pointed. The only resemblance of this creature to the *Syncarida* is the very general feature that a carapace is absent, and there is really no reason for supposing that this fossil is not a generalised Amphipod. The condition of the eyes is unknown.

(B) Segmentation.

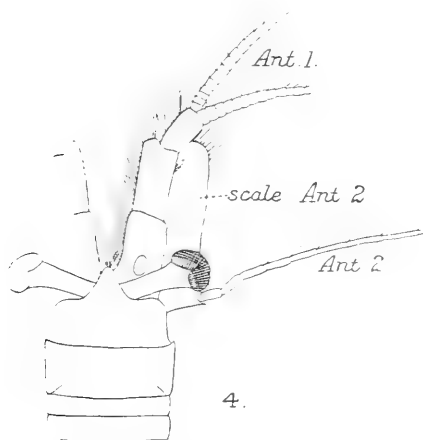
Perhaps the most striking feature of the *Anaspidacea* is the entire absence of a carapace. In *Anaspides tasmaniae* there appear to be eight free thoracic segments and there are undoubtedly six abdominal segments, without counting the telson. The true segmental value of the first thoracic segment behind the head has, however, been called in question by Calman (8). He inclines to the view that the groove separating the head from what appears to be the first segment corresponds to the cervical suture in the *Decapoda*, and that the apparent segment behind this suture really represents three segments belonging to the two pairs of maxillæ and the first thoracic limbs. The compound nature of the segment is possibly indicated by the definite lateral suture which crosses it on each side in the position shown in text-figs. 1 and 4.

Whether this anterior segment represents three fused segments or not, a question which may remain open to doubt, it is certain that its freedom from the head is far more complete in *Anaspides* than in any of the higher *Malacostraca*, and that the process of cephalisation has not gone so far in *Anaspides* as in the latter. Since, also, there is no doubt that the first thoracic segment is incorporated in, though it may not entirely represent, this segment, we will speak of it as the first thoracic segment.

The segmentation of *Paranaspides* corresponds exactly with that of *Anaspides*, but in *Koonunga cursor* there are only seven free thoracic segments, the anterior segment bearing the first thoracic limbs being definitely fused with the head, so that the segmentation agrees with the condition in the more primitive *Amphipoda* and *Isopoda*.

Among the fossil *Anaspidacea* we meet with an interesting condition. In *Præanaspides* there are seven large thoracic segments, and an extremely narrow segment in front, sepa-

TEXT-FIG. 4.



Paranaspides lacustris. Head with first and second antennæ in situ.

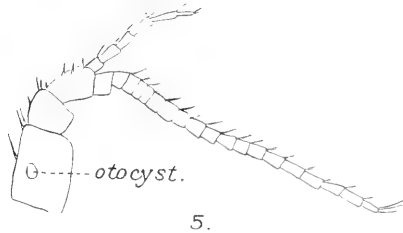
rated from the head by a distinct groove (text-fig. 3). In *Palæocaris*, *Gampsonyx*, and probably *Gasocaris* there are also eight thoracic segments, the most anterior segment behind the head being narrow as in *Præanaspides*. The extreme narrowness of this segment suggests that it really does represent the single first thoracic segment which in *Anaspides* has invaded the head-region, and finally in *Koonunga* has become fused definitely with the head. Behind this rather problematical first segment the segmentation of the body agrees perfectly in all the *Anaspidacea*, both

living and fossil. There is no trace of an extra segment in the posterior part of the thorax, which has been supposed to be present in the Euphausiidae.

(c) Appendages.

The first antennæ in all the Syncarida present a very uniform structure; there is a three-jointed peduncle with two flagella attached. In all the living forms, and probably in *Præanaspides*, there is a definite flexure between the second and third joints; this flexure does not appear in the fossil *Gampsonyx* and *Palæocaris*. In all forms, except apparently *Gampsonyx*, the inner flagellum is very much

TEXT-FIG. 5.



Koonunga cursor. First antenna.

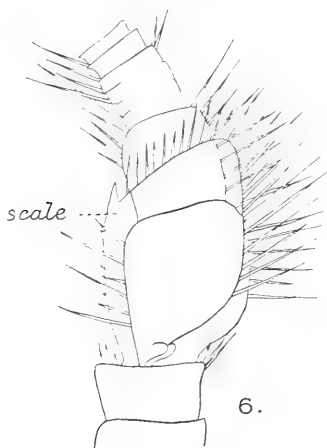
shorter than the outer; in *Gampsonyx* the two flagella appear to have been of equal length.

In all the living forms an auditory organ has been discovered upon the upper surface of the basal joint of the peduncle of the first antenna. This organ is roughly oval in *Paranaspides* and *Koonunga*, kidney-shaped in *Anaspides*.

It consists of a hollow sac opening on the dorsal inner surface of the basal joint of the first antenna by a narrow transverse slit. The hollow of the sac is filled with fluid, but there are no solid concretions of any kind. On the outer wall of the sac is a row of club-shaped chitinous rods, arranged in a single antero-posterior series. If we study the histology of the sac by means of a transverse series (Pl. 12, fig. 1) we see

that the club-shaped rods are fixed into hollow sockets by means of a pedicel which is continuous into one of the columnar cells which form the outer wall of the otocyst. From these cells muscular strands pass outwards and are connected with the pigmented ectodermal cells upon the outermost wall of the antenna. The internal chitinous wall of the otocyst is furnished with short tooth-like setæ. Below these setæ are flattened cells which come into connection with fine nervous processes sent out from the nerve of the first antenna.

TEXT-FIG. 6.

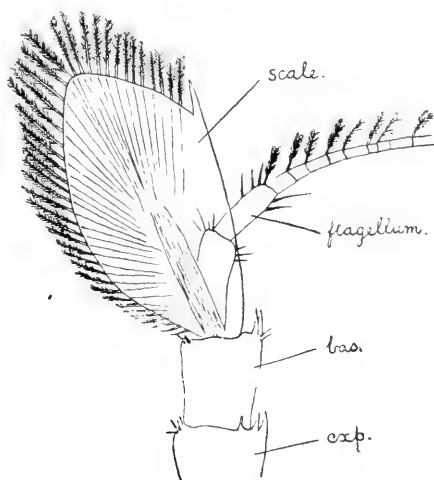


Anaspides tasmaniae. Second antenna.

The way in which this otocyst functions is not very obvious. It is clear that the club-shaped rods are not the final sense elements since they are not connected directly with the nerve-endings. The final sense elements are evidently represented by the short setæ on the internal wall, which have not been hitherto observed. It appears, therefore, that the club-shaped rods transmit the stimulus through the fluid of the sac to the sensory setæ on the internal wall and so to the nerve. The muscular apparatus connecting the club-shaped rods with the external ectoderm suggests that the original stimulus comes from the exterior, and

impinges on the ectodermal cells of the antenna, then that these transmit the stimulus to the muscles which pull upon

TEXT-FIG. 7.



Paranaspides lacustris. Second antenna. *bas.* Basipodite.
cxp. Coxopodite.

TEXT-FIG. 8.



Koonunga cursor. Second antenna.

the club-shaped rods. These in their turn transmit the impulse to the setæ and so to the nerve.

If this interpretation is correct the otocyst of the *Anaspi-*

dacea, although agreeing essentially with that of the Decapoda, differs from the latter in responding to stimuli from the external world, as well as to internal stimuli set on foot by the position of orientation.

The second antennæ consist of a two-jointed protopodite, which supports an exopoditic scale and a flagellate endopodite. The scale is comparatively small in *Anaspides* (text-fig. 6)

TEXT-FIG. 9.

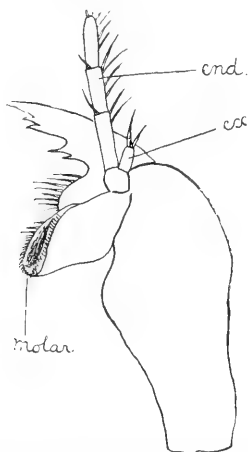
*Anaspides tasmaniae*. Mandible.

and the fossil forms; it is considerably enlarged in *Paranaspides* (text-fig. 7), probably in correlation with the swimming habit, and is altogether absent in *Koonunga* (text-fig. 8). The typical form of this antenna with its exopoditic scale and flagellum is characteristic of the "Schizopoda" and Decapoda.

The mandible in *Anaspides tasmaniae* (text-fig. 9)

has the biting face divided into three regions—an upper toothed portion, which differs slightly in the right and left mandible, a lobe bearing a row of spines, and a lower molar surface. The lacinia mobilis, characteristic of the Peracarida, is absent. The palp is three-jointed. The mandible of *Paranaspides lacustris* (text-fig. 10) has the same structure as the above, but the palp has a peculiar formation, which is particularly well marked in old specimens. In old specimens it appears to be distinctly four-jointed, and the basal joint carries a very definite, little, external branch

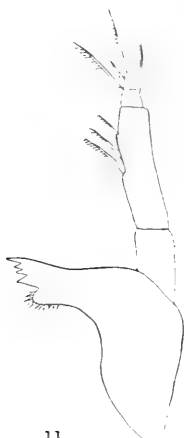
TEXT-FIG. 10.

*Paranaspides lacustris*. Mandible.

tipped with two setæ. In young specimens the extra joint, i. e. between segment two and three, may be absent, and the external branch is not so conspicuous. The external branch occupies the position of an exopodite, and if the mandibular palp in this form is really biramous, it would be unparalleled in Crustacea except among the Copepoda and Ostracoda. Considering, however, that *Paranaspides* is otherwise a rather specialised form, and that the character in question is best marked in old specimens, it seems doubtful if we are really dealing with a primitive characteristic.

The mandible of *Koonunga* (text-fig. 11) possesses a toothed ridge and a single lobe beneath it bearing short

TEXT-FIG. 11.

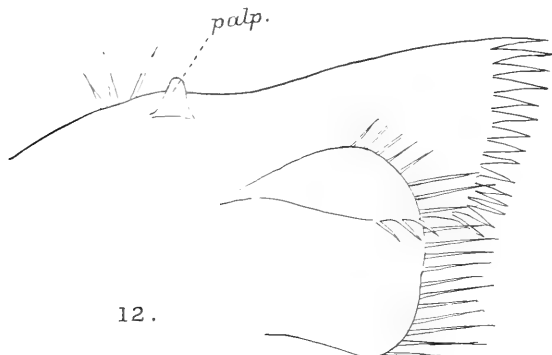


11.

Koonunga cursor. Mandible.

spines and a few small papillæ. Sayce regards this lower lobe as the molar surface, the spine row being, according to

TEXT-FIG. 12.



12.

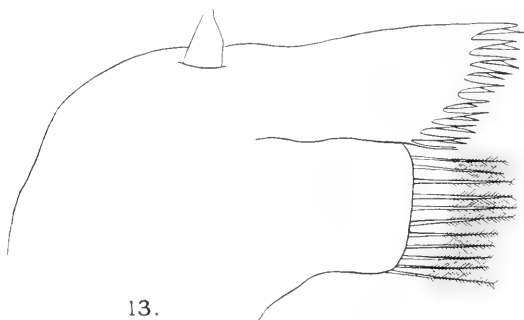
Anaspides tasmaniæ. First maxilla.

him, absent, but since the molar surface is not normally furnished with setæ in this manner, it seems better to regard

the lower lobe as corresponding to the spine row of the other forms. The palp is three-jointed, with a very short terminal joint.

Apart from the abnormal condition of Koonunga, the

TEXT-FIG. 13.



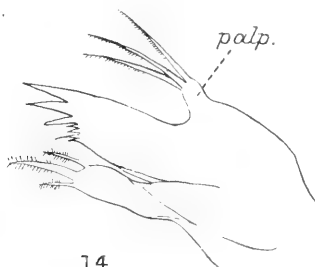
13.

Paranaspides lacustris. First maxilla.

mandible of the Anaspidacea resembles that of the Mysidacea, except that the lacinia mobilis, characteristic of the latter, is absent in the former.

In the Euphausiacea and Decapoda the mandible consists

TEXT-FIG. 14.



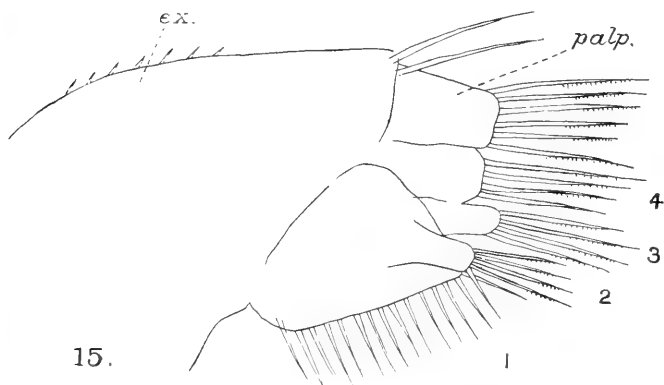
14.

Koonunga cursor. First maxilla.

of biting lobe and molar surface without any spine row or lacinia mobilis. The Anaspidacean mandible is therefore intermediate in structure between that of the Peracarida and Eucarida.

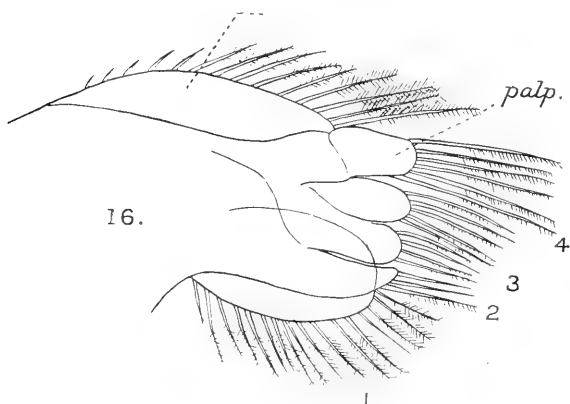
The first maxilla of *Anaspides* (text-fig. 12) consists of two biting blades, the upper one armed with stiff spines, the

TEXT-FIG. 15.



Anaspides tasmaniae. Second maxilla. *ex.* Exopodite.
1, 2, 3, 4. Gnathobasic lobes.

TEXT-FIG. 16.



Paranaspides lacustris. Second maxilla. *ex.* Exopodite
1, 2, 3, 4. Gnathobasic lobes.

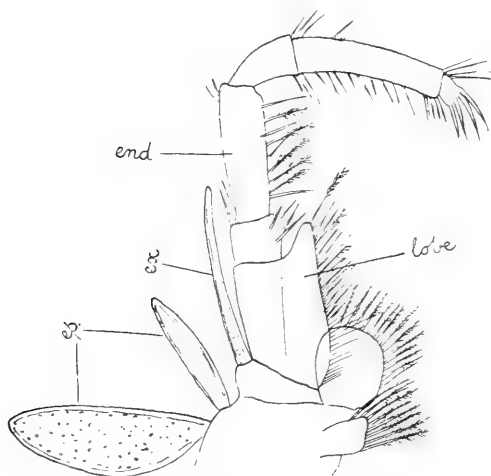
lower with plumose setæ; a palp is present in the form of a small conical tubercle. In *Paranaspides* the structure is essentially similar, but the palp is rather more conspicuous. In

Koonunga the palp is still more developed, and the lower biting blade is reduced, being tipped with only three setæ.

The second maxilla (text-figs. 15, 16, 17) has a very uniform structure in the three genera. It may be interpreted as consisting of four gnathobasic lobes, a palp which has taken on the function of a gnathobase, and an exopoditic lobe, which is well developed in *Paranaspides*, much reduced in *Anaspides*, and absent in *Koonunga*.

The structure of this maxilla resembles that of the Mysi-

TEXT-FIG. 19.



Paranaspides lacustris. First thoracic limb.

dacea more closely than that of the Euphausiacea, especially in the distinctness and arrangement of the gnathobases.

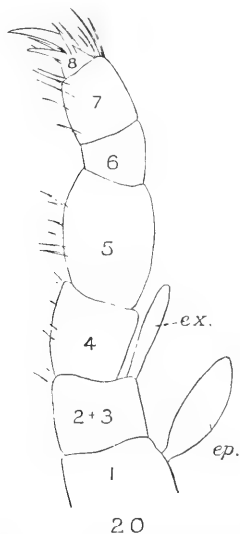
Among the fossil forms the structure of the mandibles and maxillæ has not been elucidated.

The first thoracic limb (text-figs. 18, 19, 20) differs only in detail from those of the following segments. It consists of a pediform endopodite with a reduced lamelliform exopodite. In *Anaspides* and *Paranaspides* the limb is composed of eight distinct segments, a protopodite of two joints and an endopodite of six. The coxopodite in both forms

bears a pair of delicate leaf-like gills externally, while internally, i. e. towards the mouth, two gnathobasic lobes are developed. The limb is flexed between the fifth and sixth segments, so that there are three segments distal to the "knee-joint" and five proximal to it. The terminal segment is short, and carries, as in all the thoracic limbs of the Anaspidacea, three enlarged setæ, of which the central one is the largest.

In *Paranaspides* the first joint of the endopodite (i. e.

TEXT-FIG. 20.



20
Koonunga cursor. First thoracic limb.

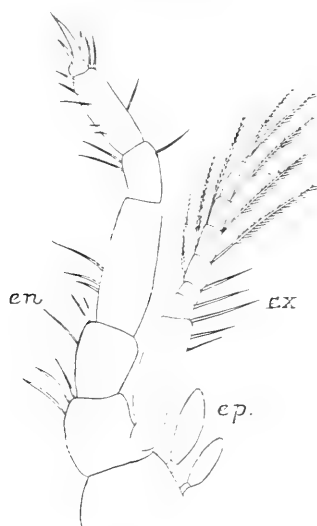
propodite) is expanded towards the mouth to form a definite lobe.

In *Koonunga* the structure of this limb is aberrant. The coxopodite is entirely without gnathobases. There are only seven segments in the limb, and since there are three segments distal to the "knee-joint" it is clear that a segment has disappeared from below, i. e. proximal to, this joint. There is no doubt that what has happened is that the propodite or first joint of the endopodite has fused with the basi-

podite, a process which can be observed to occur in some of the posterior limbs of Anaspides. If this is so, it is clear that the exopodite no longer springs from the basipodite, as it normally should do, but from the fused basipodite and propodite.

The second to the sixth thoracic limbs may be treated together, as they only differ in unimportant details. As a

TEXT-FIG. 21.



21.

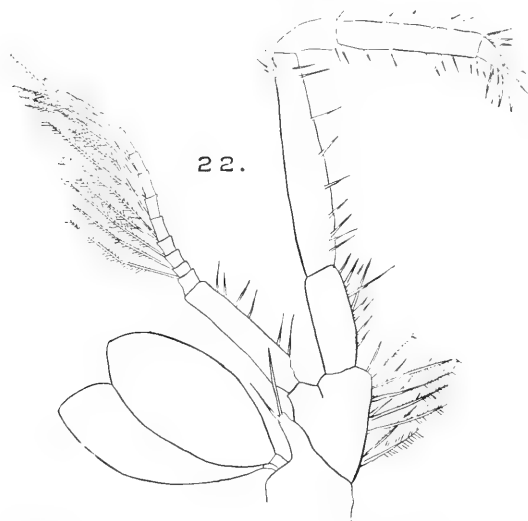
Koonunga cursor. Second thoracic limb. *ex*. Exopodite. *en*. Endopodite. *ep*. Gills.

type we may describe the fourth thoracic limb of *Paranaspides* (text-fig. 22). This limb is composed of a pediform endopodite and a flagellate exopodite borne on a two-jointed base. The coxopodite bears a pair of gills. We see in this limb the process of fusion of the basipodite with the propodite, the joint between them being represented by a groove which does not completely traverse the fused segments. In the more anterior limbs and especially in young specimens of both *Anaspides* and *Paranaspides* the

groove may be complete. In the second thoracic limb of *Koonunga* (text-fig. 21), and indeed, in all the thoracic limbs of this form, the fusion of the basipodite and propodite is complete, so that the limb appears to be constantly formed of seven joints instead of eight. In all cases, nevertheless, there are constantly three segments above the "knee-joint."

In the female sex of all the three genera, the coxopodite of the fifth, sixth and seventh limbs bears on its internal

TEXT-FIG. 22.



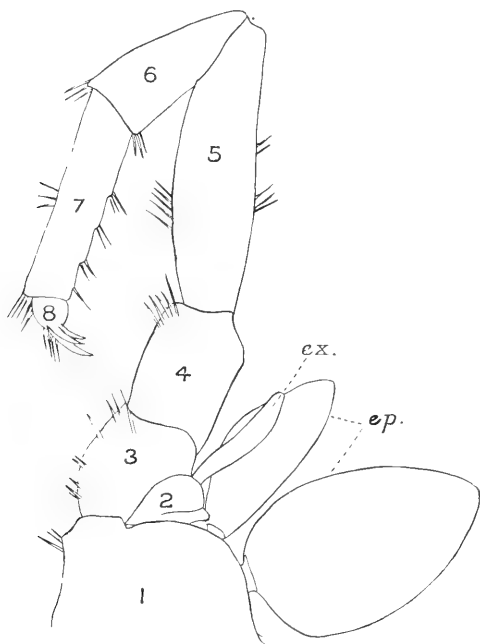
Paranaspidetes lacustris. Fourth thoracic limb.

face a small setose lobe (text-fig. 24). The function of this lobe, which is entirely confined to the female sex, is unknown, but since the lobes are only present in the hinder limbs in the neighbourhood of the oviducts and spermatheca, it may be suggested that they assist the process of fertilisation in some way. The openings of the oviducts in the female are situated on the coxopodites of the sixth pair of limbs (text-fig. 27).

The seventh thoracic limb differs from the foregoing in that in *Anaspides* and *Paranaspidetes* the exopodites are

reduced to small unsegmented lamellæ, while in *Koonunga* they are absent altogether. In *Anaspides* the limb is eight-jointed; the basipodite, carrying the exopodite, is very small, but separated from the propodite by a distinct groove. In *Paranaspides* this groove is very incomplete, while in *Koonunga* there is no groove at all, the two segments being

TEXT-FIG. 23.



23.

Anaspides tasmanica. Seventh thoracic limb of male.

entirely fused. Attention has already been called to the presence of the setose lobe on the coxopodite of this and the two preceding limbs in the female sex.

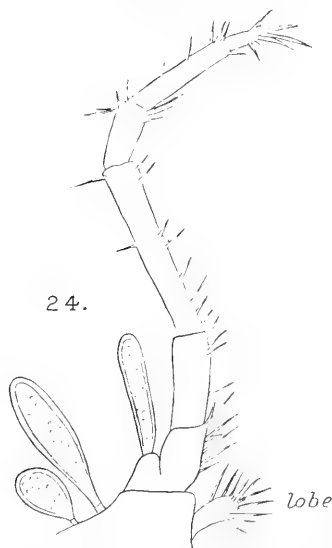
The eighth thoracic limb (text-fig. 25) in all three living genera is composed of apparently seven joints, the second and third segments having no doubt, from the analogy of the preceding limbs, fused together. The exopodite has

completely disappeared and there are no gills attached to the limb.

In the male the openings of the vasa deferentia are situated on the inner edges of the coxopodites of the eighth thoracic appendage (text-fig. 26).

In the females of the three living genera a very conspicuous spermatheca is to be seen, placed between the last pair of thoracic limbs in the ventral middle line (text-fig. 27). It consists of a large conical papilla with a single median opening

TEXT-FIG. 24.

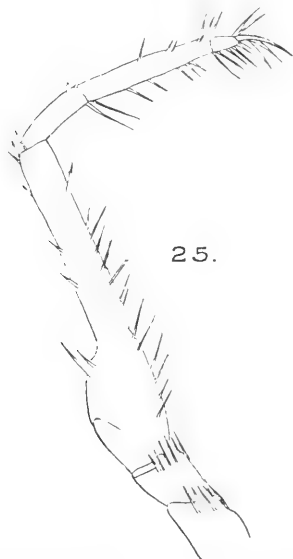


Paranaspides lacustris. Seventh thoracic limb of female.

which leads into a wide tube. The tube bifurcates into two slightly ramifying passages in which spermatozoa may frequently be found. The tube and its passages are lined with chitin secreted by a definite epithelium, and round the ramifying bifurcations a cellular tissue is aggregated of a connective or supporting character, probably with much the same function as cartilage. This tissue is also found in the labrum, and its peculiar histological character is shown on Pl. 12, fig. 17.

The presence of this spermatheca is of considerable taxonomic importance, as it appears to be entirely absent in the other Schizopods, viz. Mysidacea and Euphausiacea, but to

TEXT-FIG. 25.

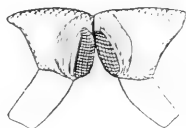


25.

Paranaspides lacustris. Eighth thoracic limb.

be present in certain of the more primitive Decapods. In the lobster and certain prawns a similar spermatheca is

TEXT-FIG. 26



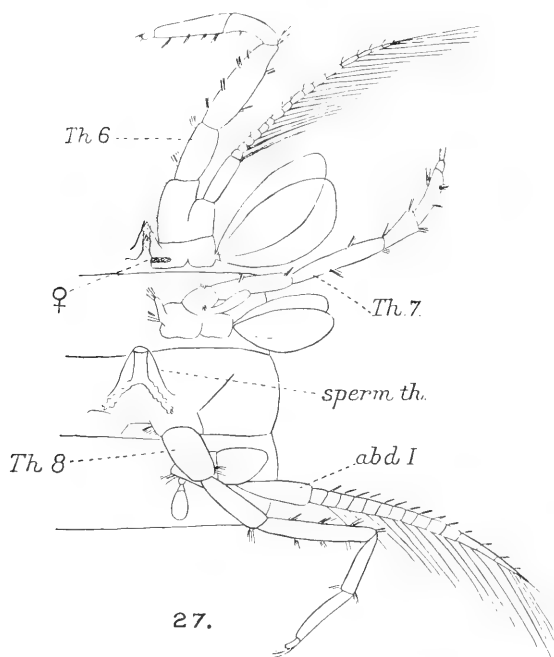
26.

Paranaspides lacustris. Basal segment of eighth thoracic limbs, showing male openings.

present, and in the peculiar Eryonidea (*Polycheles*, *Willemoesia*) the presence of a spermatheca in the same position was pointed out to me by Mr. Gray, of the Oxford

University Museum. The investigation of this spermatheca in the female of *Polycheles* has revealed a structure identical with that of *Anaspides*. The spermatheca of *Polycheles* is a shield-shaped chitinous structure with a median opening leading into a tube which bifurcates exactly as in *Anaspides*.

TEXT-FIG. 27.



Paranaspides lacustris, ♀. Ventral view of the last three thoracic appendages and first abdominal of left side in situ. *Th. 6.* Sixth thoracic limb. *Th. 7.* Seventh. *Th. 8.* Eighth. *Abd. I.* First abdominal. *Spermth.* Spermatheca. ♀. Female opening.

There can be no doubt that the structure in both cases is strictly homologous, and that we have in the spermatheca of *Anaspidacea* a Decapodan character, parallel to the presence of the otocyst on the first antennæ.

The thoracic limbs of the fossil *Syncarida* may now be dealt with as far as they are known, and those of *Præanas-*

pides (text-fig. 3), as being the best known, will receive first attention. The first thoracic limb is unknown. The second limb was apparently composed of three or four small basal joints and an expanded segment below the "knee," and probably three segments above the "knee." Except that no exopodite can be seen, it appears to have agreed with the corresponding limb of the living Anaspidacea. The succeeding thoracic limbs agree very perfectly with those of living forms. There was a two-jointed protopodite from which sprang a flagellate exopodite and a stout five-jointed endopodite, three of these segments being distal to the knee as in living Anaspidacea. In the last two thoracic limbs it is impossible to make out an exopodite, and this is again in agreement with the structure of the living genera. In the most perfectly preserved limbs it is only possible to make out seven segments in each limb, so that the fusion of the second and third segments may have already taken place in this form, but it is more probable that there were eight segments and that the condition of preservation does not permit us to see them all.

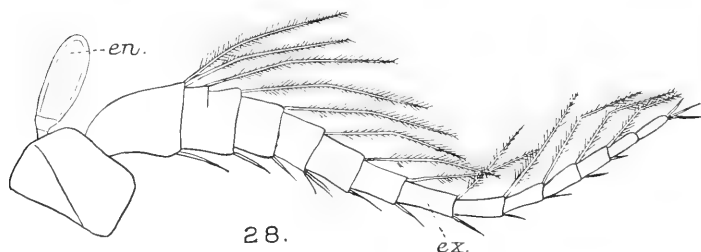
In *Gampsonyx* and *Palæocaris* we can only obtain a vague idea of the structure of the limbs. In the former the second limb was apparently of a raptorial nature, being greatly enlarged and furnished with prominent spines; the succeeding limbs one can only describe vaguely as biramous. In *Palæocaris*, if we can trust the diagrammatic reconstruction of Packard (text-fig. 56), the last six thoracic limbs were all similar and all biramous, with stout endopodites and slender exopodites.

In *Gasocaris* the endopodites are all similar and stoutly built, but Fritsch denies the presence of exopodites at all, a denial about which we may suspend our judgment, owing to the delicacy of the exopodites in the Anaspidacea and the difficulty of making them out even in the best preserved fossils.

The abdominal appendages, 1-5 in the females of *Anaspides* and *Paranaspides*, have all a very similar structure, except the fifth, which is without an endopodite. The

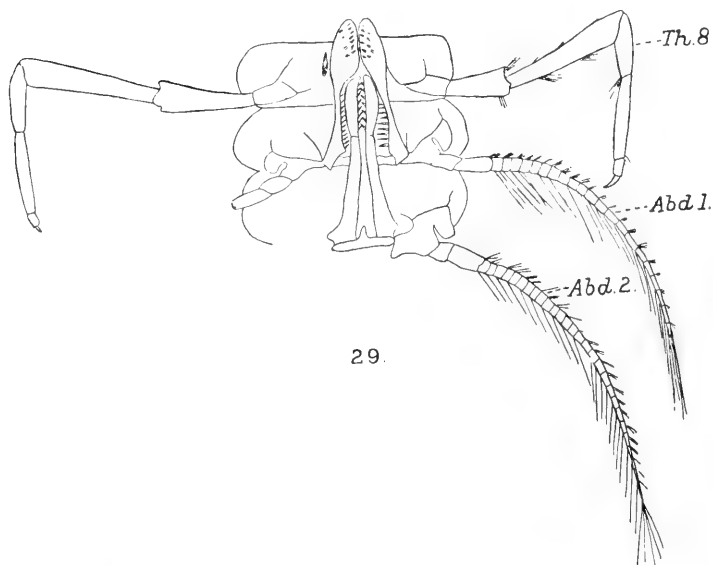
limb consists of an expanded protopodite of two segments, from which springs a long setose and many-jointed exopodite

TEXT-FIG. 28.



Paranaspides lacustris. Third abdominal appendage.
En. Endopodite. *Ex.* Exopodite.

TEXT-FIG. 29.



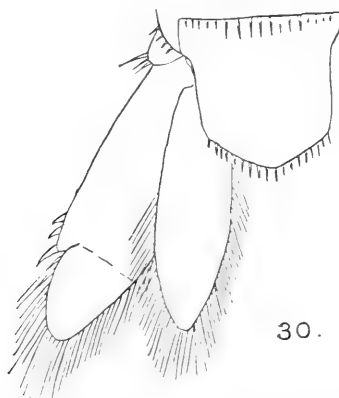
Paranaspides lacustris. First two abdominal appendages of male in ventral view in situ. *Th. 8.* Eighth thoracic limb.
Abd. 1. First abdominal. *Abd. 2.* Second abdominal appendage.

and a very small flabellate endopodite. This endopodite is absent on all the first five abdominal appendages of *Koonunga*.

In the males the endopodites of the first two pairs of abdominal limbs are curiously modified as copulatory styles (text-fig. 29). They are large, hollow, club-shaped organs, well armed with setæ and hooks, disposed in a characteristic pattern, and the endopodites of the second segments fit into hollow spaces excavated in the first in a piston-like manner. The exopodites of these limbs are of a normal form.

The presence and structure of these copulatory styles is a distinctly Eucaridan feature, recalling similar structures in the Euphausiacea and Decapoda.

TEXT-FIG. 30.

*Anaspides tasmaniae*. Telson and uropods.

The abdominal limbs of *Præanaspides* show a long setose exopodite, much as in the living forms; the endopodite was apparently much reduced, as in living *Anaspidae*. The reconstruction of *Palæocaris* also shows uniramous abdominal appendages, so that they also apparently agreed well with the living forms. The vague reconstruction of *Gampsonyx* shows us apparently biramous limbs with the two branches of equal length, but it is very likely that the long setæ on the exopodites have been confused with endopodites, an error that might occur in reconstructing *Præanaspides*.

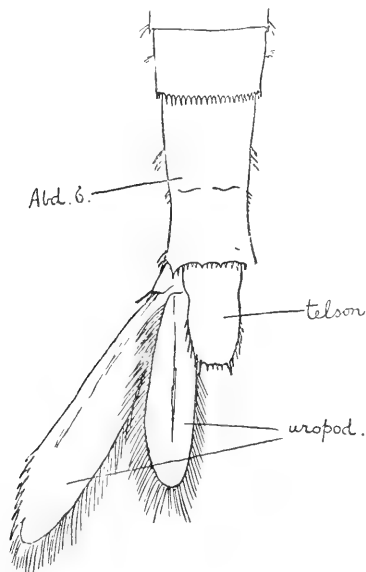
The sixth abdominal limbs or uropods and the telson

have very characteristic forms, which are depicted in text-figs. 3, 30, 31, 32, 33, 55, 58, 61.

It will be noted that the uropods and telson of *Paranaspides* are more elongated than in the other genera, in correlation with the swimming mode of life.

Special attention must be called to the close correspondence between the uropods of *Præanaspides* (text-fig. 3) and *Anaspides* (text-fig. 30); even individual setæ, e.g. those

TEXT-FIG. 31.



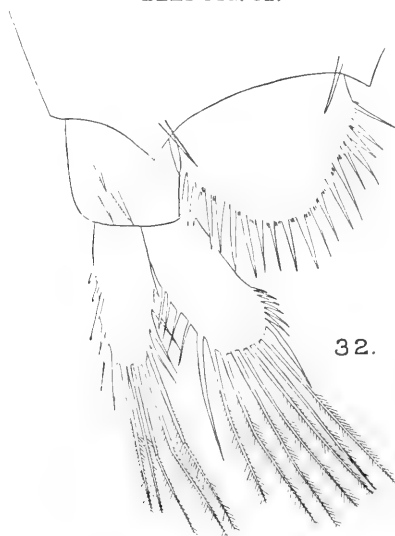
Paranaspides lacustris. Telson and uropods.

on the outer border of the exopodites, being practically identical in the two cases. The short setæ also upon the margin of the last two segments in *Anaspides* and *Paranaspides* are also represented in the fossil. The detailed correspondence in these and the other limbs of *Anaspides*, *Paranaspides* and *Præanaspides* permits us to place them with confidence in the same family. The uropods and telson of *Gampsonyx*, *Palæocaris* and *Gasocaris* agree on the whole very accurately with the

other members of the Syncarida. The uropods and telson of *Acanthotelson* appear to have possessed a very aberrant form.

Attention may again be called to the observation of Professor Fritsch, that in *Gasocaris* and *Gampsonyx* an oval swelling is present near the base of the inner ramus of the uropod, which he interprets as an otocyst (text-figs. 55, 61). Nothing of the sort is to be observed in any of the living

TEXT-FIG. 32.



Koonunga cursor. Telson and uropods.

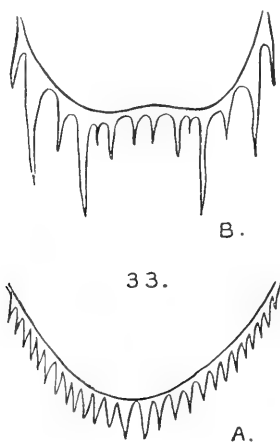
Anaspidacea nor in *Præanaspides*. If this structure really is an otocyst we are evidently dealing with a character which must have belonged originally to the Syncarida and the primitive Eumalacostraca, and has been retained in certain Peracarida (Mysidacea) but lost in the higher Syncarida, Peracarida and Eucarida.

(D) Theoretical Considerations.

A theoretical consideration of the appendages of the Syncarida with their associated organs will bring out several

points of importance. The nature of these structures is plainly of a generalised and primitive character. In the first place all the appendages, with the exception of the first antennæ, are either typically biramous or have departed very little from the biramous plan. In the next place it is impossible, on the character of the appendages, to place the Anaspidacea in either the Peracarida or Eucarida, or any other division of the Malacostraca. The mandible is of a Peracaridan

TEXT-FIG. 33.



End of telson. A. In *Anaspides tasmaniae*. B. *Paranaspides lacustris*.

type, but lacks the lacinia mobilis; the maxillæ are possibly nearer those of the Peracarida than of the other divisions, but a palp is still present on the first maxilla and the exopodite of the second maxilla is greatly reduced.

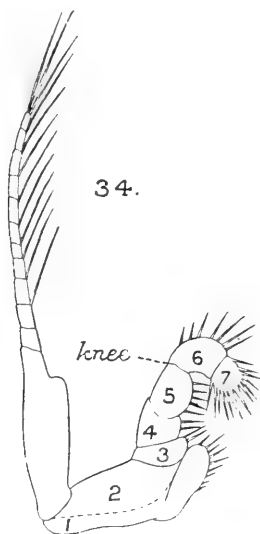
On the other hand the otocyst on the first antennæ is paralleled only by the Decapoda, as is also the spermatheca at the base of the last thoracic limbs, while the copulatory styles are also Eucaridan.

The segmentation of the thoracic limbs is also very interesting as affording us a primitive condition from which that of the Peracarida on the one hand and that of the Eucarida on the other can be severally derived. We have seen that

the primitive Anaspidacean condition of these limbs is the possession of eight segments or joints, of which three are placed distally to the "knee" while five are placed proximally (e. g. text-fig. 18).

In the Peracarida and Eucarida we observe typically seven segments, but the "knee" in the two divisions, as pointed out by Hansen (14), is in a different position. In the Peracarida (e. g. Mysidacea) (text-fig. 34) there are two

TEXT-FIG. 34.



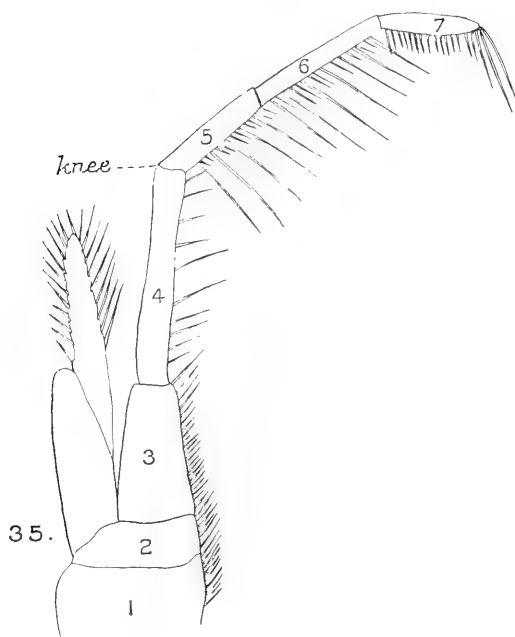
First thoracic appendage (maxillipede) of *Macromysis flexuosa*.

segments distal to the "knee," and primitively five proximal to it; in the Eucarida there are three segments distal to the "knee" and four proximal to it (text-fig. 35). Now we have seen that in the Anaspidacea there is a tendency for the second and third segments to fuse, and this process carried to completion would give us the Eucaridan limb. In the Peracaridan limb we may suppose that a segment has disappeared distal to the knee, probably the terminal segment, which in the Anaspidacea is very small.

In this manner it would appear that the "knee-joint" in Peracarida and Eucarida is homologous, while a different segment has been suppressed in each case, in the Peracarida the terminal segment, and in the Eucarida the second segment from the body, which has fused with the third.

Hansen has suggested that the claw usually present on the terminal joint of the Peracaridan limb represents the lost

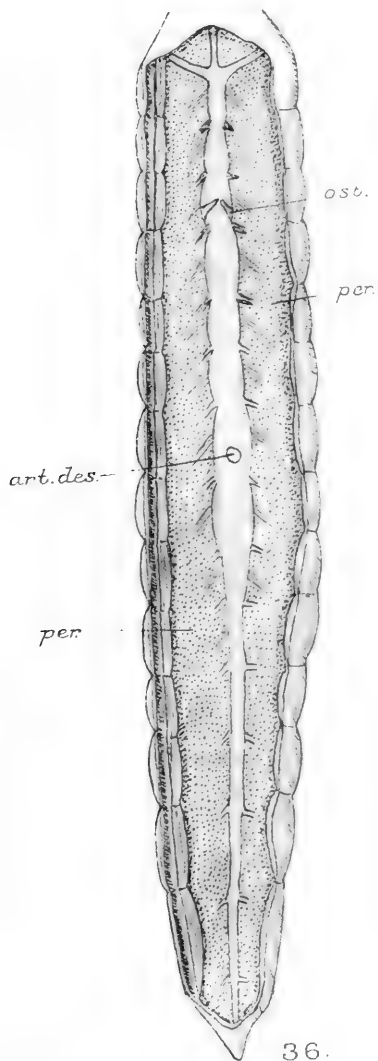
TEXT-FIG. 35.

First thoracic appendage of *Nyctiphanes*.

terminal segment, and this may possibly be the case, though a theoretical refinement of this nature must always remain doubtful.

However this may be, the Anaspidacean limb with its eight segments gives us the generalised condition from which the Peracaridan and Eucaridan types can be easily deduced.

TEXT-FIG. 36.



Anaspides tasmaniae. Dissection from dorsal surface, to show heart and pericardium (*per.*). *Ost.* Ostium. *Art. des.* Place of origin of descending or sternal artery from the heart.

3. INTERNAL ANATOMY (*Anaspides tasmaniae*).

(A) Pericardium, Heart and Vascular System.

If a median dorsal incision be made in *Anaspides*, and the skin and dorsal muscles be turned away on each side, we find that we are in a spacious division of the body cavity with a very definite pigmented floor, which stretches from the first to the last segment. This space is the pericardium and its floor is pierced laterally in each segment by lacunar spaces which lead down into the cavities surrounding the bases of the appendages. The heart, which lies in the pericardium, is a long contractile tubular structure with an anterior expanded portion stretching from the first to the eighth thoracic segment; in the first abdominal segment it narrows considerably and is continued into a vessel which appears to be contractile, but is perhaps more rightly to be considered as a posterior dorsal aorta. The heart and aorta are fixed to the floor of the pericardium by a series of intersegmental short muscles, while the heart in the thoracic region is also supplied with segmental dorsal alary muscles. The heart is constricted intersegmentally where the ventral muscles attach it to the floor of the pericardium, but ostia are only present in one place, namely at the base of the third thoracic segment, where there appears to be a single pair.

Anteriorly the heart gives off three arteries, a median ophthalmic artery and paired antennary arteries.

In the seventh thoracic segment a sternal artery leaves the heart, and running obliquely downwards enters a ventral artery in the sixth thoracic segment, which runs forwards and backwards just dorsal to the nerve cord (text-fig. 44, *art. vent.*). A small subneural vessel is also present.

The elongated tubular heart is of a strictly Peracaridan type, and recalls very strongly the heart of the *Mysidæ*; the arterial system is of a generalised Malacostracan nature. The dorsal muscles which form the roof of the pericardium

are arranged in four lateral longitudinal bands constricted intersegmentally.

The blood-corpuscles appear in sections as oval cells of varying size, but generally with similar nuclei. Above and to the sides of the cardiac division of the stomach there is a conspicuous mass of tissue with crowded darkly staining nuclei and without definite cell outlines, in which very numerous mitoses may be observed, even in an adult fully-grown animal. At the edges of this tissue, which lies free in the hæmocœl, cells can be seen to be detaching themselves which have the appearance of blood-corpuscles. This tissue (Pl. 12, fig. 2) is present in the same position in "Schizopoda" and Decapoda which I have examined, and there appears to be little doubt that it constitutes the blood-forming organ of the higher Crustacea in which the blood-corpuscles are reproduced.

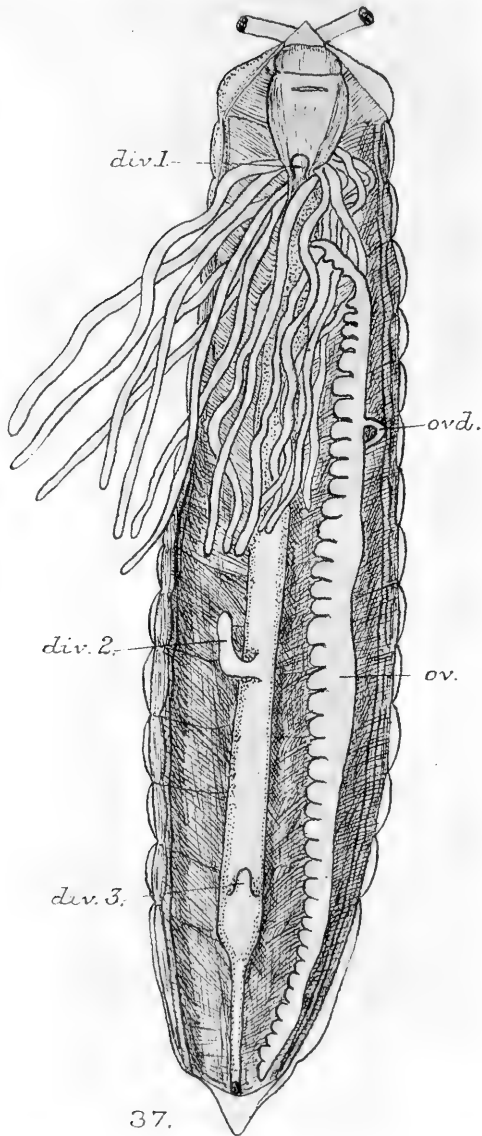
(B) The Alimentary Canal.

On removing the heart and the floor of the pericardium we find the alimentary canal with its associated glands. We will first shortly enumerate the various parts of the alimentary canal, beginning from in front backwards.

The short dorso-ventrally directed œsophagus leads into an expanded stomach which is furnished with a series of complicated setose ridges. At the pyloric end of the stomach a short median dorsal diverticulum marks the position where the mid-gut or endodermal portion of the alimentary canal begins and the stomodæum ends. At the same point, but ventrolaterally, a great number of long, slender, liver cæca enter the stomach, to the number of about thirty.

The mid-gut is continued downwards as a straight tube until the second abdominal segment, where a large and conspicuous dorsal diverticulum is given off. This diverticulum, which is of a glandular nature, belongs to the mid-gut, but it does not mark the place where the proctodæum begins. This position is marked by a third diverticulum in the fifth abdo-

TEXT-FIG. 37.



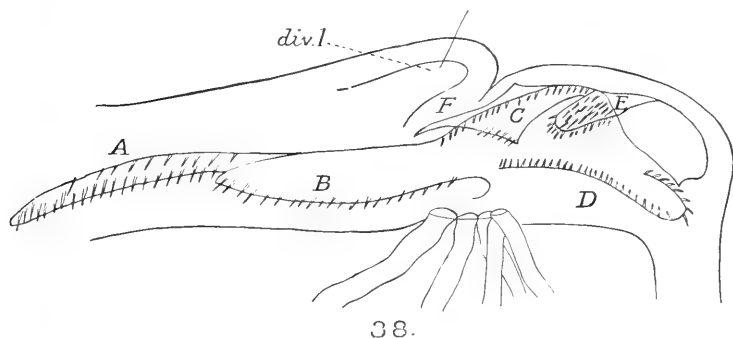
Anaspides tasmaniae. Dissection from dorsal surface, heart and pericardium removed, to show ovary (*ov.*) on one side and alimentary canal. Left ovary removed. *Ovd.* Oviduct. *Div. 1.* First dorsal diverticulum. *Div. 2.* Second. *Div. 3.* Third.

minal segment, which also belongs to the mid-gut, but immediately below it the character of the epithelium entirely changes, and a chitinous lining, marking the proctodæal invagination, covers the internal surface of the intestine. The anus opens ventrally on the telson.

We will now describe these various portions in more detail.

The stomach may be divided into an anterior cardiac and a posterior pyloric portion. The division between them is marked dorsally by the first diverticulum of the alimentary

TEXT-FIG. 38.



Anaspides tasmaniae. Stomach removed from body and viewed laterally. For lettering see text.

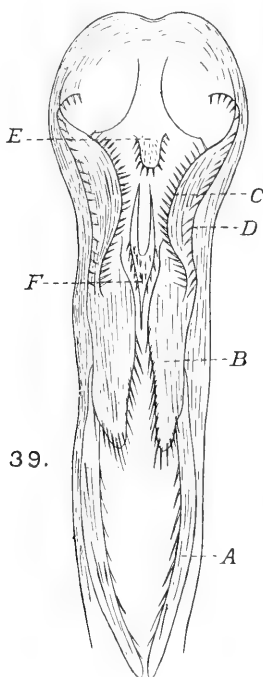
canal, and ventrally by the entrance of the liver cæca, so that the cardiac portion is stomodæum while the pyloric is endodermal, although chitinous pieces of ectodermal origin are projected into the pyloric division.

The cardiac portion of the stomach is furnished with a pair of lateral elevations carrying setæ. Each lateral elevation is in the form of an incomplete circle, the setæ being present in two main pieces of the ridge, viz. *c* and *d* in text-figs. 38, 39, 40. There is also present a median setose prominence (*e*), and a more posteriorly placed tooth (*f*) which projects into the pyloric cavity. In the median ventral line there is a prominent pad (*h*) (text-fig. 40) which projects

into the cavity of the stomach and stretches into the pyloric portion (see also transverse section, Pl. 12, fig. 3).

The pyloric portion of the stomach is furnished with a pair of very long setose ridges on each side, the hindermost ridge of each side being produced backwards far down the intestine (A and B, text-figs. 38, 39, 40).

TEXT-FIG. 39.



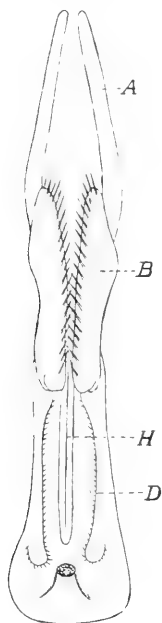
Stomach from dorsal surface, showing chitinous ridges, etc.

In comparing this armature of the stomach of *Anaspides* with other Malacostraca, I make use especially of Gelderd's useful work on the digestive system of the Schizopoda (15).

The lateral pieces of the cardiac division (C, D) correspond to Gelderd's ridges s_1 , which he finds to be present in all Malacostraca; similarly the lateral pieces of the pyloric division (A, B) correspond to his ridges s_2 , which are also

universally present. The median pieces (E and F) correspond to the urocardiac ossicle and median tooth of the Decapoda. The piece (E) is present in most Malacostraca, but the posterior piece (F) possibly points to Decapodan affinities. The great length of the lateral pyloric pieces (A and B) in *Anaspides* resembles the condition of the Euphausiidae and not that of the

TEXT-FIG. 40.



40.

Stomach from ventral surface.

Peracarida, in which these pieces are short. Again, in the Peracarida there is a complicated median ventral ridge in the pyloric division, which in the Euphausiidae is simpler and appears to be absent in the *Anaspides*, while the elongated ventral ridge (H) in the cardiac division of *Anaspides* recalls closely that of the Euphausiidae. We may therefore conclude that in those points in which the gastric mill is not merely Mala-

costracan, it inclines to the Eucaridan rather than the Peracaridan type.

The first dorsal diverticulum to the gut is a thick-walled pocket which opens into the pyloric division of the stomach by two lateral passages on the outside of the lateral pyloric ridge (B). In the section shown in Pl. 12, fig. 4, only one lateral opening is seen on the right side.

The histological character of this diverticulum is extremely puzzling (Pl. 12, fig. 4). It consists of thick walls in which crowds of nuclei are densely packed without definite cell-outlines round them, and many of the nuclei are seen to be either actually undergoing mitosis or else in preparation for division. This process of mitotic division is to be observed in fully grown adult specimens, so that we are evidently dealing with a tissue which remains in a permanently embryonic condition. There are no cells of a glandular nature in this diverticulum, as might well be expected from its position on the alimentary canal.

It is impossible to do more than guess at the function of this remarkable organ, but from the active reproduction of the cells composing it, it may be suggested that its function is to supply new epithelial cells for the lining of the alimentary canal as the old cells wear out and become effete.

Laterally the walls of the first diverticulum pass into the epithelium of the mid-gut or endoderm (Pl. 12, fig. 5). The portion of the mid-gut lying between the first and second diverticula is essentially glandular in nature, especially in the anterior and dorsal region. In this region the epithelial cells are tall and columnar. The majority of these cells have lightly-staining oval nuclei, but wedged in between these ordinary columnar cells are nests of much-flattened cells with spindle-shaped nuclei, which stain intensely with hæmatoxylin. These cells are apparently special gland-cells of some kind, which pour a secretion into the gut. Ventrally and posteriorly the lining epithelium of the mid-gut is composed of short cubical cells with rather darkly staining nuclei.

The basement membrane of the mid-gut is remarkably

thick and conspicuous; and it is thrown into marked sinuosities of outline; this membrane stops abruptly when the mid-gut passes over into stomo- or proctodæum. Exteriorly to the basement membrane is a thin submucous layer with flattened darkly-staining nuclei (Pl. 12, fig. 5).

The second dorsal diverticulum of the mid-gut is by far the largest. Histologically (Pl. 12, fig. 6) it is simply formed as a pocket from the dorsal mid-gut epithelium, and its cells are elongated and columnar with very numerous nests of flattened special gland-cells. There can be no doubt about the function of this diverticulum; it is simply a digestive gland which pours its secretions into the alimentary canal.

The remaining portion of the mid-gut lying between the second and third diverticula is of a simple structure, and is evidently chiefly of an absorptive nature (Pl. 12, fig. 7). The epithelium is composed of short columnar cells with striated outer borders; the basement membrane, characteristic of the mid-gut, is still to be observed, while the submucosa forms a thick reticular layer in which large homogeneously staining nuclei are embedded. There are no gland-cells in this region.

The third dorsal diverticulum is exceedingly small, and is composed of columnar cells and numerous nuclei embedded in a common protoplasm. There are no special gland-cells. A fair number of mitoses can always be observed in this diverticulum, though not so many as in the first diverticulum; its function may be similar to that suggested for the first, viz. to keep up a supply of epithelial cells for the lining of the alimentary canal. The intestine behind the third diverticulum is proctodæum, being lined internally with chitin, which is thrown into numerous folds. The epithelium is columnar and hyaline, being very similar to that of the stomodæum.

It remains to describe the liver. This organ is composed of very numerous slender tubes, as many as thirty being often present, which open ventrally into the pyloric division

of the stomach by a wide common opening. The histological character of these tubes varies greatly according to the condition of metabolism. A transverse section through the middle of a tube, when the animal is starved, shows a regular lining of tall columnar cells, the majority of which have hyaline, reticular cytoplasm, staining pink with eosin, and granular nuclei (Pl. 12, fig. 8). These cells are mainly absorptive in function. Scattered among these cells are narrower cells with rather coarsely granular cytoplasm, which is darkly coloured with hæmatoxylin. These cells are special gland-cells and probably secrete a ferment.

At the ends of the tubes the nuclei are greatly crowded, but both kinds of cells can be recognised.

After feeding heavily, the histology of the tubes changes (Pl. 12, fig. 9). The special gland-cells are no longer recognisable, and the absorptive cells lose their definite cell-outlines and are distended with oily globules. Certain of the cells consist merely of an envelope containing an immense vacuole with a darkly staining nucleus flattened on one side. The liver of *Anaspides* therefore has, at least, two distinct functions; it produces digestive ferments which are poured into the stomach, and it also plays an important part in the absorption and storing of assimilated material.

If we compare the alimentary tract of *Anaspides* with that of other Malacostraca we see that the structure of the stomach points rather to Eucaridan affinities. The presence of dorsal diverticula at the juncture of endodermal mid-gut with stomodæum and proctodæum is a Decapodan character, since in the "*Schizopoda*," i.e. *Euphausiidæ* and *Mysidæ*, etc., there is never a diverticulum between mid-gut and proctodæum. We have seen that there is also another diverticulum in the middle of the mid-gut, and this character is, as far as we know, peculiar to *Anaspides* and its immediate allies.

The liver, although in certain respects peculiar, is nearer to the Eucaridan plan than to the Peracaridan, since in the latter there is typically present a glandular ridge upon which the

gland-cells are concentrated, while in the Eucarida and Anaspides the gland-cells are distributed about among the absorptive cells.

On the whole, therefore, the alimentary tract of Anaspides, while showing certain peculiar features, points to Eucaridan and especially Decapodan affinities.

(c) Excretory System.

The excretory organ of Anaspides is situated at the base of the second maxilla; it is a maxillary gland. We can distinguish four chief regions: (1) A straight excretory duct with rather thick walls and darkly staining somewhat flattened nuclei (Pl. 12, fig. 10). This duct passes into the base of the second maxilla on each side and opens by a pore on the external border of the appendage. (2) The excretory duct passes internally into a coiled excretory tube with striated walls and greatly flattened darkly staining nuclei (Pl. 12, fig. 11). (3) This coiled tube passes insensibly into another coiled tube with an epithelium of a more glandular nature and with oval nuclei containing granules of chromatin (Pl. 12, fig. 12). The cytoplasm of these cells is more granular but has a faintly striated appearance. (4) The glandular tube is coiled into an expanded sac, the end-sac into which it opens. The end-sac is lined with a flattened epithelium, the cells of which contain globules of a yellowish colour (Pl. 12, fig. 13).

The presence of a maxillary gland, and the entire absence of an antennary gland, is only found elsewhere in the Malacostraca among certain Isopods and in *Nebalia*. It is unknown either in the "Schizopoda" or Decapoda.

(d) Reproductive Organs.

Female.—The external sexual characters of the female, together with the spermatheca, have been described (pp. 516–518). The ovary of an adult Anaspides is a lobed

structure stretching from about the second thoracic segment to the extreme hind end of the abdomen (text-fig. 37). If we take a horizontal section through the ovary (Pl. 12, fig. 14) we see that the lobes contain small immature ova while the external part of the tube is filled with large, nearly mature ova filled with a purplish yolk. The external wall of the lobed portion consists of small undifferentiated cells of the germinal epithelium. The inner wall of the ovary consists of cells, most of which contain large nuclei which stain of an uniform dark colour with hæmatoxylin, while there is present a number of granules which stain deep black. These cells may be called the trophic cells. On their inner borders they are vacuolated, and it is evidently their function to elaborate food material, which they supply in the form of yolk to the developing ova. A certain number of these trophic cells can be seen lying among the eggs in the middle of the tube. The oviducts are simple straight tubes lined with short columnar cells; they pass below the ventral muscles to open on the coxopodites of the sixth thoracic limbs. They are not supplied with any accessory glands.

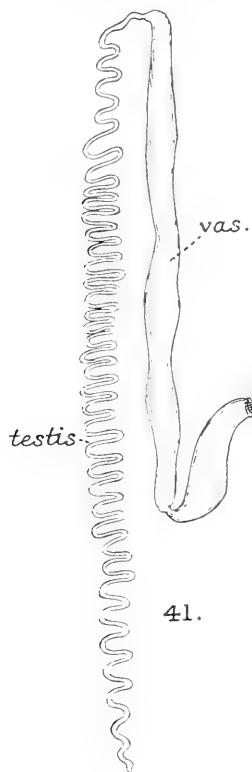
Male.—The testes (text-fig. 41) are coiled tubes running from the anterior thoracic region to the extreme hind end of the body. At the anterior end of the coiled tube a duct passes posteriorly, and then turning anteriorly again opens at the bases of the eighth thoracic limbs.

In the glandular part of the testis of a young *Anaspides* we can study the process of spermatogenesis (Pl. 12, fig. 15). We see the primary and secondary spermatocytes undergoing mitosis, and fully-formed spermatozoa. On the outside of the tube we see large cells with darkly staining nuclei of an exactly similar appearance to the trophic cells found in the ovaries. These cells are not, however, to be observed in an adult testis. In an adult testis we only see nests of spermatocytes in various stages of spermatogenesis, or else groups of fully formed spermatozoa.

The upper part of the descending duct is sterile, so far as the production of spermatozoa is concerned, and it is formed

purely of trophic cells (Pl. 12, fig. 16). The lower portion of the duct has a thick epithelial wall with oval granular nuclei, and these cells produce an albuminous material that is cast into the lumen of the duct and solidifies round the

TEXT-FIG. 41.



Anaspides tasmaniæ. Testis and vas deferens of one side.

spermatozoa to form the spermatophores. There is a thick muscular layer surrounding the lower part of the vas deferens. In the section (Pl. 12, fig. 16) the top of the spermatophor, with its albuminous coat, is cut across.

The spermatozoa are filiform elongated bodies with a con-

spicuous globular head and a long flagellum (text-fig. 42). They resemble closely the spermatozoa of all the Peracarida.

The spermatophores are horseshoe-shaped bodies about half an inch in length, with a constriction near the middle. They possess a fine chitinous investment on the outside, as well as

TEXT-FIG. 42.

Spermatozoon of *Anaspides tasmaniae*. \times about 50.

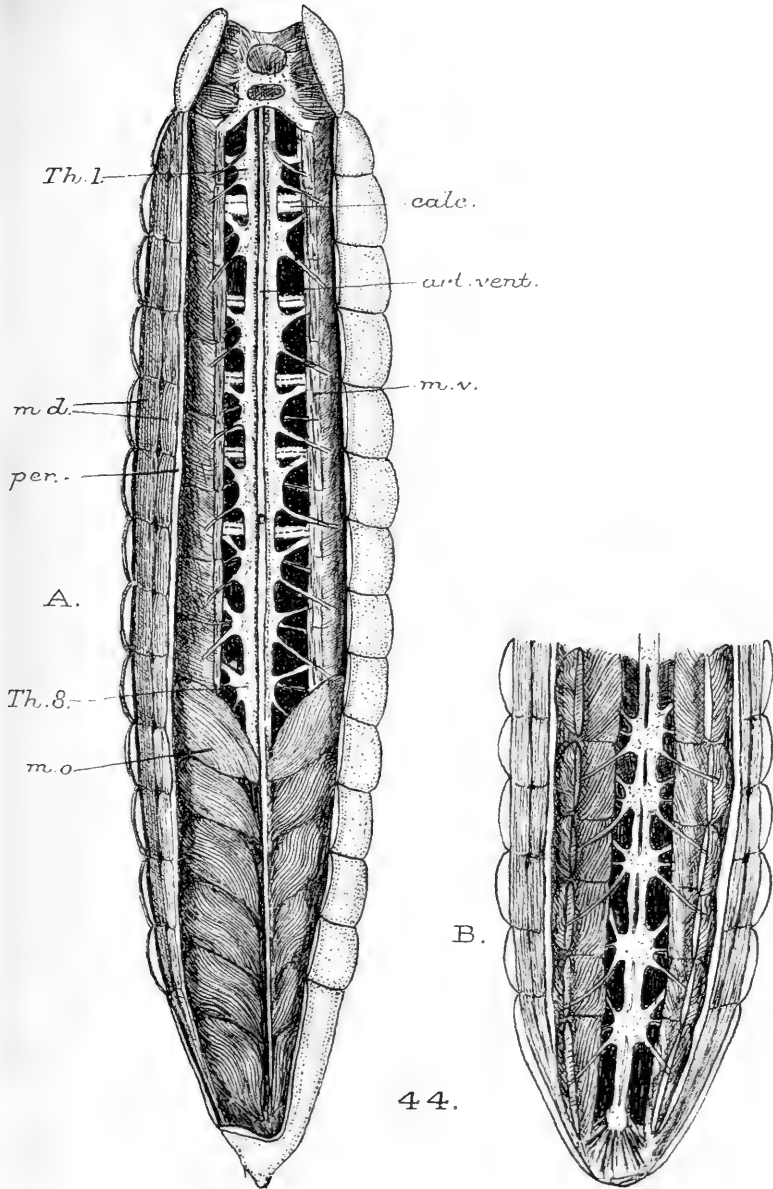
the albuminous material within. They are placed by the male into the spermatheca of the female, where they may be sometimes found protruding their horn-like ends to the

TEXT-FIG. 43.

Spermatophores of *Anaspides tasmaniae*. \times 3.

exterior. After the spermatozoa have passed out of them into the spermatheca they soon drop off.

The filiform character of the spermatozoa is a Peracaridan character; the presence of definite spermatophores is Eucaridan.



44.

Anaspides tasmaniae. Alimentary canal and gonads removed to expose nervous and muscular system. A. With abdominal oblique muscles (*m.o.*) in situ. *Th.1.* First thoracic ganglion. *Th. 8.* Eighth thoracic ganglion. *calc.* Calcareous band between ganglia. *art. vent.* Ventral artery. *m.v.* Ventral muscles. *m.d.* Dorsal muscles, cut through and turned back. *per.* Edge of pericardium, which has been removed. B. After removal of oblique muscles to show six abdominal ganglia.

(E) Muscular System.

The dorsal muscles which lie above the pericardial space form four dorso-lateral, segmented, longitudinal bands running the entire length of the body. The oblique muscles are segmented bundles running obliquely downwards in each segment; they are very much larger in the abdominal segments than in the thoracic. Segmentally arranged ventral bands are also present, which again are larger in the abdominal than in the thoracic region. In order to see them and the nerve-cord in the abdomen, the oblique muscles have to be removed.

(F) Nervous System.

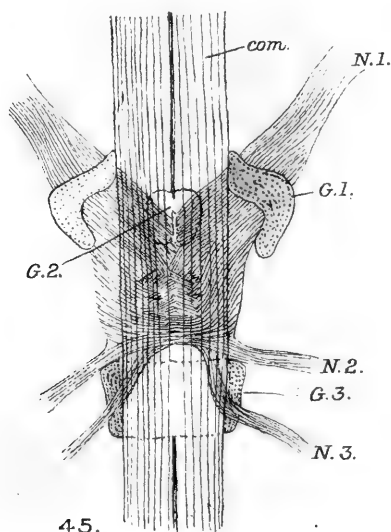
We will deal first with the nerve-cord behind the sub-oesophageal ganglion. There are in the thoracic region eight distinct ganglionic thickenings, one for each free thoracic segment: similarly in the abdomen there are six ganglia (text-fig. 44).

There are five bands of calcareous concretions situated between the first six thoracic ganglia (*calc.* text-fig. 44). This is the only place where lime is present in the whole of the body.

Each thoracic ganglion gives off three chief pairs of nerves: an anterior thick pair which pass forwards to the appendages of the segment; a more posterior slender pair which appear to supply the ventral muscles of each segment; and a still more posterior pair which innervate the oblique muscles. If we examine a single thoracic ganglion more carefully by means of sections, we may obtain a diagrammatic reconstruction, such as is shown in text-fig. 45. The inter-ganglionic commissures (*com.*) are seen to fuse above and below the ganglionic area, but their fibres are continuous right through that area on the dorsal surface. The large nerves (*N. 1*) to the appendages send their fibres ventral to the commissural

fibres, and in the ganglion they form thick bundles of transverse commissures which anastomose in the thick fibrous region in the ventral middle line. A ganglionic mass is applied to the dorsal surface of this nerve (*G. 1*) and also to the ventral (*G. 2*). The two nerves to the muscles are seen issuing more posteriorly from the ganglion; their fibres also lie ventral to the commissural longitudinal fibres. A

TEXT-FIG. 45.



Reconstruction of a thoracic ganglion of *Anaspides tasmanica*. *com.* Longitudinal commissures of cord. *N. 1.* Nerve to appendage. *N. 2.* Nerve to ventral muscles. *N. 3.* Nerve to oblique muscles. *G. 1.* Dorsal ganglionic mass. *G. 2* and *3.* Ventral ganglionic masses.

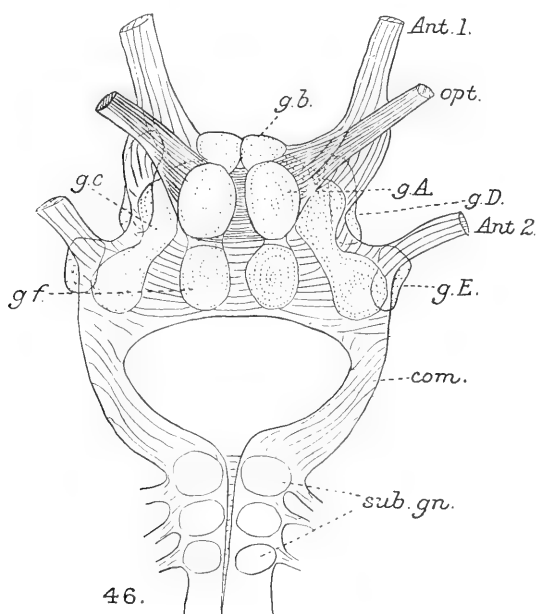
ganglionic mass (*G. 3*) is situated only on the ventral surface at the exit of these nerves.

The brain or supra-oesophageal ganglion gives issue to three nerves, the optic (*opt.*), antennulary (*Ant. 1*), and antennary (*Ant. 2*) nerves.

The optic nerves spring from the dorsal surface of the brain, being the only nerves with this position of origin.

Where they enter the brain there is a dorsal ganglionic mass (text-fig. 46, *g. A.*). The dorsal ganglionic masses (*g. C.*) belonging to the antennary nerves are applied anteriorly to the ventral root of the optic nerve, and it is possible that this part of the ganglionic mass (*g. C.*) really belongs to the optic nerve. If this were so, the optic nerve would be supplied with a dorsal

TEXT-FIG. 46.



Reconstruction of brain of *Anaspides tasmaniae*. *Ant. 1.* Antennular nerve. *Ant. 2.* Antennary nerve. *Opt.* Optic nerve. *g. A.* Dorsal ganglionic mass applied to root of optic nerve. *g. F.* Its posterior continuation. *g. B.* Ganglion applied to root of *Ant. 1.* *g. C.* Ganglion applied dorsally to root of *Ant. 2.* *g. D.* Ganglion applied ventrally to root of *Ant. 1.* *g. E.* Ganglion applied ventrally to root of *Ant. 2.* *Com.* Peri-oesophageal commissures. *sub. gn.* Suboesophageal ganglion.

and ventral ganglionic mass, as in the case of a regular trunk nerve. Lying dorsally in the brain there is a bundle of transverse commissural fibres forming a regular optic chiasma.

The antennular nerves (*Ant. 1.*) enter the brain ventrally

and are supplied with a dorsal (*G. B.*) and ventral (*G. D.*) ganglionic mass on each side. Their fibres form thick transverse longitudinal bands in the ventral part of the brain, with complicated branches passing posteriorly. The antennary nerves (*Ant.* 2) enter the brain laterally and ventrally, and are supplied with a very large dorsal ganglionic mass (*G. c.*), which passes anteriorly underneath the fibres of the optic nerves, and a smaller ventral mass (*G. E.*). The fibres which enter the brain from these nerves form a very complicated and massive system, occupying the whole of the posterior part of the brain.

The peri-œsophageal commissures (*com.*) are short, and ventrally to the œsophagus they come together in the sub-œsophageal ganglion (*sub. gn.*), which gives off three nerves to the mandible and two pairs of maxillæ.

(G) Theoretical Considerations.

If we pick out the more important points in the internal anatomy of Anaspides, in order to compare it with the other Malacostraca, we perceive that its internal anatomy is of a generalised type resembling in some respects the Peracarida and in others the Eucarida, especially the Decapoda.

The chief Peracaridan features are the elongated, tubular heart and the filiform spermatozoa, while the possession of a maxillary gland is paralleled by certain Peracarida (Isopoda). The alimentary canal, on the other hand, in so far as it is not peculiar, recalls that of the Decapoda. The nervous system is of an unconcentrated primitive character, with a discrete ganglion in each thoracic and abdominal segment.

Comparing these conclusions with those derived from a consideration of the external characters, we may observe that they are in complete agreement. From the external characters of the limbs and of the segmentation we judged that the Anaspidacea stood midway between the Peracarida and the Eucarida, but on a more generalised and primitive plane,

from which either of the two divisions might be derived. We also saw that the Anaspidacea, in their external characters, approached nearer to the Decapoda, than to the Euphausiid type of the Eucarida, and this, again, appears to be the case in the internal anatomy. This would indicate that the Euphausiacea are a late offshoot from the Decapodan stock, and if this conclusion is accepted it is evident that the old group of the Schizopoda, including the Mysidacea, Euphausiacea and Anaspidacea is an unnatural assemblage and must be abandoned (see phylogenetic tree on p. 551).

4. BIONOMICS: (HABITS, REPRODUCTION AND DISTRIBUTION).

(A) Habitat and General Habits.

Anaspides tasmaniae inhabits deep pools of rivers or tarns on the mountains of southern and western Tasmania; the water in which it lives is always absolutely clear and cold, and the animal clambers about upon the stones or among the submerged mosses and liverworts at the bottom of the pools. It very rarely swims, though it occasionally does so in a lazy fashion, and it will occasionally rise to the surface of the water and turn over on to its back in the manner of a Phyllopod. Its usual mode of progression is walking or running in the attitude presented in Pl. 11, fig. 1; when alarmed it darts forwards or sideways by powerful strokes of its abdomen and tail-fan. I never observed it to spring backwards, a movement of which it appears to be incapable.

The exopodites of the thoracic limbs are not used in locomotion to any appreciable extent, their function being to keep the water agitated round the gills and so assist in respiration. Even when the animal itself is stationary the exopodites can be seen to be in a continual waving motion.

As remarked before, the body is always held perfectly flat and unflexed whether the animal is walking, swimming, or at rest.

They appear to be omnivorous, as they will feed upon the dead bodies of insect larvæ or even upon one another, but their chief food is the algal slime covering the rocks among which they live, and they also browse upon the submerged shoots of mosses and liverworts.

The rivers and tarns in which they live are singularly free from any enemies such as predaceous fish which might prey upon them, the only fish inhabiting these highland waters being the little "Mountain Trout," *Galaxias truttaceus*. The English Trout, which have multiplied so wonderfully in the Tasmanian streams and lakes, have hardly penetrated to the mountain fastnesses where *Anaspides* dwells.

The only parasite found infecting *Anaspides* is a peculiar species of neogamous gregarine which lives in the free state in the alimentary canal and forms large associated cysts in the liver-tubes, often in very great numbers. This gregarine will shortly be described in this journal by Mr. J. S. Huxley.

Paranaspides lacustris is known only from the specimens collected by me in the great Lake of Tasmania at an elevation of 3700 ft., where it inhabits the littoral region, living among the weeds and stones at a small depth rather after the manner of a prawn.

Its markedly humped back and translucent green colour give it very much the appearance of a small prawn, e.g. Hippolyte, and it follows more of a swimming habit than *Anaspides*, but in other respects it resembles the latter closely. It doubtless falls an easy prey to the great quantities of large English brown trout which inhabit the lake, and it is probably in danger of an early extinction.

Koonunga cursor has been found hitherto only in a small runnel issuing from the Mullum Mullum Creek, Ringwood, to the west of Melbourne, and it is the only member of the living Anaspidacea which lives at a low level. In general appearance and habits it resembles *Anaspides* more closely than *Paranaspides* does, although it is morphologically very distinct. The specimens which Mr. Sayce

kindly showed to me ran about with great activity, keeping the body perfectly flat and unflexed as in *Anaspides*.

(B) Distribution.

We have seen that the living Anaspidacea are all confined to the temperate part of the Australian region, called by Professor Spenser the Bassian Subregion, where they inhabit exclusively fresh water, usually at a high elevation, where at any rate the winters are exceedingly cold. The fossil Anaspidacea, on the other hand, are, as far as we know, confined to the marine deposits of the northern hemisphere, being found in the Permian and Carboniferous deposits of Europe and North America. I have suggested elsewhere (12) that animals with this type of distribution, viz. in the north temperate hemisphere and in the Alpine regions of temperate Australia, probably have reached their present position in the southern hemisphere through South America and the submerged Antarctic Continent, and not through the tropics of Asia and Australia. Although this is little more than a tentative suggestion, it is, perhaps, justifiable to predict that living members of the Anaspidacea may still be found in the temperate fresh waters of South America or New Zealand.

(c) Breeding and Reproduction.

The breeding of *A. tasmaniae* appears to go on through the early summer months (December to April) as the pools on Mt. Wellington were continually being replenished with young of a very small size. Since there is no brood-pouch, it was of interest to establish what the female does with her eggs, especially as this is a character of great taxonomic importance. By keeping the animals in captivity it was observed that the male deposits two very large spermatophores in the spermatheca of the female. These spermatophores are large curved structures with a thin chitinous coat (text-fig. 43), and they project outside the sperma-

theca. The spermatozoa pass into the spermatheca and the spermatophores drop off. Fertilisation appears to take place in the oviducts, since in some sections of *Koonunga* shown to me by Mr. Sayce, spermatozoa could be seen lying in the basal part of the oviducts. As to how the spermatozoa reach the oviducal openings from the spermatheca there is some doubt, but it seems probable that they are assisted in this migration by the peculiar setose lobes on the internal faces of the last three pairs of thoracic appendages, which are only present in the female.

The female deposits and hides the fertilised eggs, which are of a purple colour and measure about 2 mm. in diameter, singly and not agglutinated together, under stones and among the roots of water plants (Pl. 12, fig. 3). This peculiar habit of oviposition is only found elsewhere among the Malacostraca in certain forms of Euphausiidae which may have pelagic eggs; in all other Malacostraca they are either carried in a brood-pouch (Phyllocarida and Peracarida), or else glued on to the abdominal limbs (Eucarida), or carried in a special chamber formed by the maxillipedes (Hiplocarida). Among Entomostraca the only forms which deposit their eggs are the Argulidae.

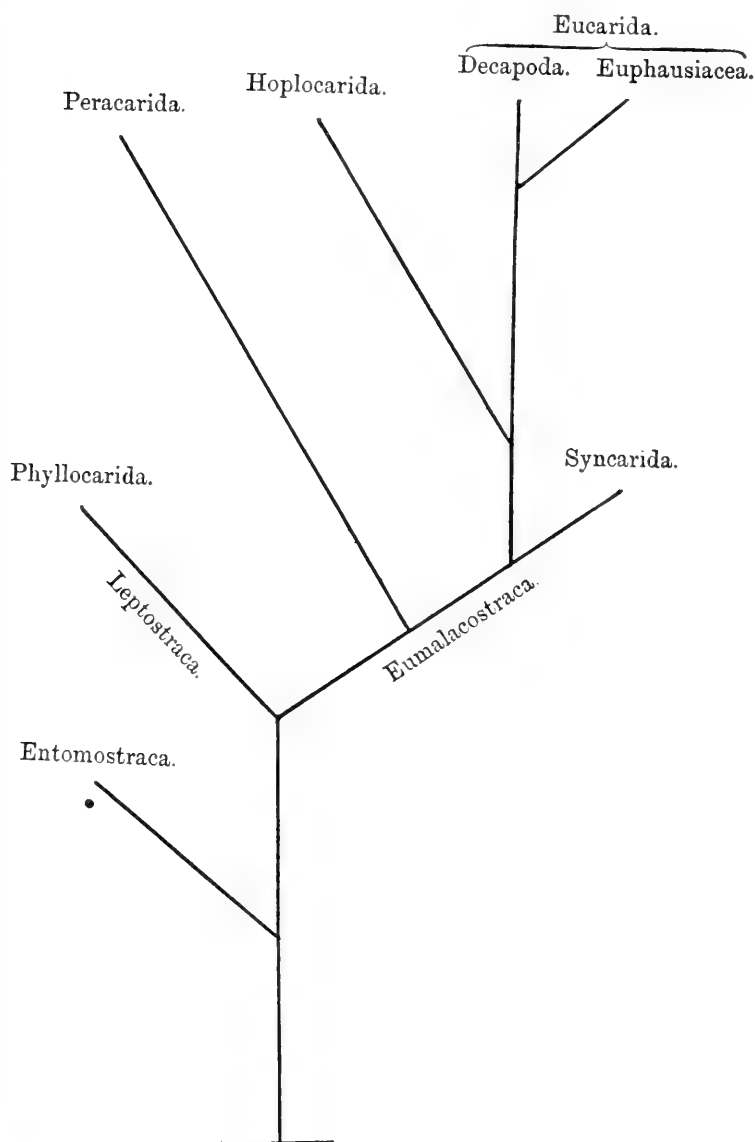
As to the development of the eggs or the presence of any larval stages my observations are unfortunately very few. I am, however, convinced that no complicated metamorphosis is passed through, as the pools under my observation were continually being replenished by minute Anaspides of 4-5 mm. in length, which already possessed the complete adult structure. It is also quite impossible that pelagic larvæ of such types as the Nauplius and Zoæa could be assumed, as they would at once be swept away in the mountain torrents down to the lowlands, where Anaspides, as a matter of fact, never occurs. It is possible, however, that the young are hatched out from the egg, not with the complete adult structure, for Mr. Sayce (10) found a minute specimen of *Koonunga* in which the abdominal appendages were incompletely developed.

5. THE RELATION OF THE SYNCARIDA TO OTHER MALACOSTRACA.

The examination which has been made in the foregoing pages of the chief characteristics of the Syncarida has convinced us that they are the most generalised group of the Eumalacostraca. We have also seen that the structure of the living representatives of the division is practically identical with that of fossils which date, at any rate, from the Carboniferous period, so that they are among the oldest Malacostraca, with the exception of the Nebaliacea, known. Their generalised and primitive nature is not only shown by their great antiquity and by the possession of such characters as the freedom of all the thoracic somites, and the presence of eight joints in the thoracic limbs, but also by the fact of their combining in their own structure many of the distinctive characters of the two divergent groups of the modern Eumalacostraca, viz. the Peracarida and the Eucarida. Thus we have seen that they possess the auditory organ, the alimentary canal and its glands, the spermatheca and copulatory organs of the Eucarida, but at the same time the structure of the heart and of the spermatozoa, the absence of a carapace, the direct mode of development and the maxillary gland point to Peracaridan affinities.

The Syncarida, therefore, represent to a great extent the ancestral form from which the Peracarida and the Eucarida have diverged. It has also been pointed out that the Syncarida are closer to the Decapoda than to the other order of the Eucarida, viz. the Euphausiacea, so that the latter, so far from being the primitive Eucaridan type, must be considered as a specialised offshoot from the main Decapodan stem, which has lost certain primitive and ancestral characters. In fact, the only primitive character retained by the Euphausiacea is the biramous structure of the thoracic limbs, and this has also been retained by the lower members of the Decapoda, and so is not particularly significant.

In the following phylogenetic scheme we have given expression to this idea.



Phylogenetic Tree, showing chief lines of descent in the Crustacea.

The position of the Hoplocarida (Squillidæ) as an early offshoot from the Decapodan stem must remain at present doubtful, but many of their characters point to this conclusion. The spherical spermatozoa, the ramified hepatic cæca, the complicated metamorphosis, the absence of a lacinia mobilis, the presence of an appendix interna on the pleopods, all suggest that the Hoplocarida have travelled some way in the Eucaridan direction since their derivation from the common Eumalacostracan ancestor. That this Eumalacostracan ancestor which diverged from the Leptostracan stock was not identical with the Syncarida must probably be conceded. But we may perhaps conceive of it as a straight-bodied ambulatory Crustacean without a carapace and with all the thoracic segments free and uncoalesced, with a tail-fan and with all its thoracic and abdominal appendages biramous. The thoracic limbs consisted of eight joints, three of these joints being distal to the "knee." It possessed stalked eyes, an antennal scale, and two flagella on the antennules, and it possibly lacked an otocyst on the antennules. Internally the heart was elongated, the alimentary canal had, perhaps, only an anterior dorsal diverticulum, and the liver-tubes were few and simple. The spermatheca were filiform and there was present both an antennary and maxillary gland. It probably had a brood-pouch as in the Phyllocarida.

From this type it was but a step to the Syncarida. Directly from this ancestral type sprang the Peracarida, with their characteristic brood-pouch; a certain amount of fusion of segments either with the head or with one another took place, and certain of them developed, independently of the Eucarida, a carapace.

The Eucarida were probably derived from an ancestor which had travelled some way in the Syncaridan direction, that is to say, it had an otocyst on the antennules, it had lost the brood-pouch, and it had developed certain other characters, such as the spermatheca and copulatory styles and a more complicated liver and alimentary canal. As it diverged from the primitive Syncarida it acquired a carapace, spherical

in place of filiform spermatozoa and a short triangular heart ; it specialised an antennary gland and it possessed a complicated metamorphosis. After its divergence it threw off the Hoplocarida and the Euphausiacea.

If this phylogenetic scheme for the Eumalacostraca be accepted it is clear that we can no longer look upon the old order "Schizopoda" as a natural assemblage standing at the base of the Eumalacostracan stock. This classification rests solely on the biramous structure of the thoracic limbs, and ignores all the other organs, whether external or internal, in which the various divisions differ so fundamentally.

We must remain in some doubt as to the presence or absence of certain characters in the ancestral Eumalacostracan which gave rise to the Syncarida, Eucarida and Peracarida. With regard to the brood-pouch it may well be that this has been independently acquired by the Leptostraca and the Peracarida, and that its absence in the Syncarida represents a primitive condition. Again, the otocyst on the antennules may have been possessed by the ancestral Eumalacostracan and lost by the Peracarida. In this connection we may mention the striking observation of Professor Fritsch, according to which an otocyst was present on the inner ramus of the uropods in the fossil Syncarida, *Gasoxaris* and *Gampsonyx*. If an otocyst was really present in this position in these forms we can only suppose that the primitive Eumalacostracan possessed it, and that it has been retained by only a few Peracarida (Mysidacea), and entirely lost by the higher Syncarida and Peracarida and by the entire division of the Eucarida.

The reconstruction of the phylogeny of the Malacostraca, therefore, leads to the reflection that the primitive ancestors of the specialised groups are not distinguished from their modern representatives so much by simplicity of structure, but rather by combining in themselves the heterogeneous elements which have been segregated out in the course of evolution and separated into the different streams of descent that have given rise to the modern groups. It was the habit

of morphologists, and perhaps still is, to imagine that a primitive ancestral form must have been simpler and have exhibited less complication of structure than its modern representatives. In pursuance of this preconceived notion the simplest organised members of a phylum or smaller group of animals was always hit upon as representing the ancestral form ; but too often it has been shown that this simplicity is the result of secondary degeneration or simplification of structure. We have only to mention the Archianelida and the Marsupials to indicate what has been the trend of morphological opinion on this subject.

It is often complained, especially by naturalists immersed in the positive details of what may appear more modern and fruitful branches of inquiry, that speculative morphology, as a reputable department of biology, is dead, killed by the wild speculations of its devotees. But it may have escaped their attention that the tendency of speculative morphology to-day is to display a more cautious temper than heretofore, and instead of attempting to link phyla with phyla by golden bridges of aërial speculation to undertake the more modest task of tracing the lines of descent within a smaller range of organisms, the history of whose evolution has been accomplished at any rate somewhere within the period of time represented by the stratified rocks.

If the family Anaspididæ was already fully differentiated in the Carboniferous period and the family Nebaliidæ in the Cambrian, it may well be conceded that to look for the ancestor of the Crustacea or to prove that *Peripatus* really links the Arthropoda and Annelida together are tasks which the cautious morphologist may well abjure. But that speculative morphology, when content to deal with the phylogenetic history of fairly confined and homogeneous groups whose fossil ancestry have been preserved for us through a long period of time, is altogether idle we need not admit. And if a general consensus of opinion might at some future time be achieved, that the process of evolution in such groups has not been effected by the gradual complication of an

originally simple structure and by the addition of new organs to comparatively undifferentiated organisms, but rather by the segregation and separation of characters originally combined in one ancestral form into the various streams of descent which have emerged from it—if this opinion might at any time be adopted and sustained, it would influence our attitude to the philosophy of evolution as profoundly as any conceptions deduced from the experimental study of living organisms, without reference to the history which they have passed through.

6. SYSTEMATIC PART.

The Anaspidacea, living and fossil, are placed in a separate division of the Eumalacostraca. Thus :

Division Syncarida (Packard 5 and 6, Calman 9).

A carapace is absent. The thoracic somites are either all distinct, or the anterior one may be fused to the head. The eyes are pedunculated or sessile. An otocyst is present on the basal joint of the antennules. The antennal protopodite consists of two segments. The mandible is without a lacinia mobilis. The thoracic limbs consist typically of eight segments, and the "knee-joint" is between the fifth and sixth segment. There are no oostegites. A spermatheca is present on the last thoracic segment of the female. There is no appendix interna on the pleopods. The endopodites of the first two pleopods of the male are modified to form copulatory styles. The branchiæ form a double series of leaf-like plates on all but the last or last two thoracic limbs. The heart is elongated and tubular. The alimentary canal is furnished with three dorsal diverticula, one at the junction of the stomodæum and mid-gut, one in the middle of the mid-gut, and one at the junction of the mid-gut and proctodæum. The hepatic cæca are numerous, elongated and unbranched, and without glandular ridges. The

excretory organ is a maxillary gland. The spermatozoa are filiform, and are transferred to the female in horn-shaped spermatophores. There is no concentration of ganglia in the thoracic or abdominal region.

The eggs are deposited immediately after fertilisation by the female and hidden singly under stones, etc.

There is no complicated metamorphosis, the young hatching out with the essential structure of the adult.

Order Anaspidacea (Calman 9).

Diagnosis of the single Order is the same as that of the Division.

Family I. Anaspididæ (Thomson 7).

The thorax is composed of eight distinct somites. The eyes are pedunculated. First antennæ of male without sensory modification. There is a well-developed antennal scale. The mandible has a cutting blade, a setose lobe and a molar expansion. The palp of the first maxilla is a non-setose papilla. The first thoracic limb has gnathobasic lobes, a slender lamellar exopodite, and two branchiæ attached to the coxopodite. The anterior thoracic limbs are clearly composed of eight segments. The last thoracic limb is uniramous. The pleopods are all biramous with a small flabellate endopodite, except the fifth pair, which are without the endopodite.

Genus 1. Anaspides (Thomson 7).

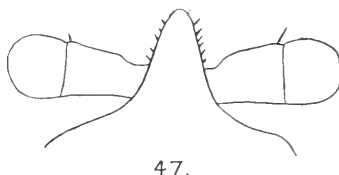
The thoracic segments are all nearly equal in length; the abdominal segments are slightly longer. The body is carried straight without any dorsal flexure. The antennal scale is shorter than the first two joints of the endopodite. The mandibular palp is without an exopoditic lobe.

The first thoracic limb has two gnathobasic lobes on the coxopodite. The sixth abdominal segment and the telson are as long as the two preceding segments. The telson is shorter than the uropods.

*A. tasmaniae*¹ (Thomson 7). Plate I, fig. 1.

The frontal margin of the head is produced into a conical projection, the sides and extremity of the cone being furnished with setæ (text-fig. 47). The eye-stalks do not project greatly beyond the lateral margins of the head. The body is carried flat and unflexed. On the head segment a median triangular piece is marked out by shallow grooves. The head segment is slightly longer than the first thoracic segment. The first thoracic segment is distinguished by the presence of two lateral sulci. The succeeding segments of both thorax and

TEXT-FIG. 47.



Anaspides tasmaniae. Head and eyes.

abdomen are sub-equal in length, but the sixth abdominal segment is considerably longer.

The first antennæ have the three basal joints short and stout; the internal flagellum consists of about twenty segments.

The otocyst is kidney-shaped.

The second antenna has a short scale, not reaching the top of the second joint of the peduncle.

The palp of the mandible is without an exopoditic lobe on its basal joint. The terminal segment is nearly half as long as the last but one.

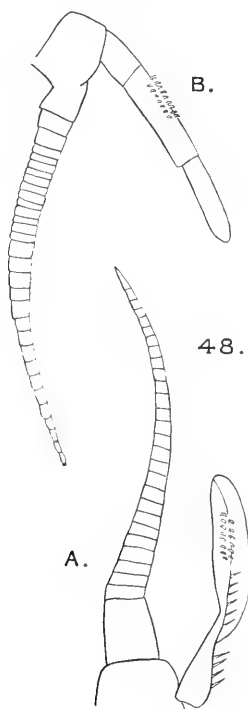
The first maxilla has a small palp and three setæ near it.

¹ In the description of the species, those characters which have been mentioned or described in the diagnoses of the genera or families or in the anatomical part are only lightly touched upon, or in some cases not mentioned.

The second maxilla has the exopoditic lobe greatly reduced and fringed with a few minute setæ.

The first thoracic appendage has two gnathobasic lobes attached to coxopodite. The terminal segment, as in all living Anaspidacea, carries three enlarged setæ, of which the middle one is the largest.

TEXT-FIG. 48.



Anaspides tasmaniae, ♂. A. First abdominal limb. B. Second abdominal limb.

There are two gills attached to the coxopodite of this limb, and of all the succeeding thoracic limbs except the last.

The seventh thoracic limb bears a small exopodite upon a distinct segment of the limb.

The fifth, sixth and seventh thoracic limbs of the female bear a small setose lobe on the inner face of the coxopodite.

The first abdominal appendage of the male possesses a spatulate endopodite furnished with a row of setæ proximally on its ventral internal face, and a pad of recurved hooks distally. The tip of the endopodite is simple.

The second abdominal appendage of the male possesses a biarticulate endopodite furnished on its internal face with a pad of recurved hooks. This pad is situated considerably below the joint separating the two segments of the endopodite. The terminal segment of the endopodite is without setæ or spines. There is no median spine on the sternum of this segment. There is a row of rather long setæ on the posterior dorsal margins of the fifth and sixth abdominal segments. The margin of these segments has a moniliform ornamentation owing to the bases of the setæ being raised.

The telson has the form shown in text-figs. 30 and 33A. It has a row of short setæ confined to the posterior border.

The uropods have a short basal segment with a few lateral setæ. The exopodite has three enlarged lateral spines on its upper external margin. The setæ fringing the uropods are uniform in size and structure.

The adult animal may attain two inches in length.

The ground colour is straw yellow, but the skin contains a great number of black chromatophores disposed in a regular pattern.

Occurrence.—In isolated pools on and near the top of Mount Wellington, Tasmania; in the pools of the upper reaches of the North West Bay River on Mount Wellington, above the Wellington Falls; in the tarns on Mount Field, on the Harz Mountains and on Mount Read, West Coast of Tasmania. At an elevation of 2000 to 4000 feet.

Genus 2. *Paranaspides* (Smith 12).

The first thoracic segment is longer than the two succeeding segments put together; the abdominal segments are much longer than the mid-thoracic segments. The body has a distinct dorsal flexure, the first abdominal segment project-

ing dorsally as a hump. The antennal scale is longer than the first two joints of the endopodite. The mandibular palp has a distinct setose exopoditic lobe. The first thoracic limb, besides the two gnathobasic lobes on the coxopodite, has the inner face of the first segment of the endopodite expanded into a setose lobe.

The sixth abdominal segment and the telson are together longer than the three preceding segments.

The telson is shorter than the uropods.

P. lacustris (Smith 12). (Plate 11, fig. 2; text-fig. 1.)

The frontal margin of the head is produced into a conical projection, the cone being tipped with a bunch of setæ. The eye-stalks project considerably beyond the lateral margins of the head (text-fig. 4). The body is carried with a marked dorsal flexure. A triangular piece is not obviously marked out on the head segment, and the head segment is equal in length to the first thoracic. Lateral sulci are present on the first thoracic segment. The first thoracic segment is equal in length to the three succeeding segments. The abdominal segments are all longer than the mid-thoracic segments. The first antennæ have the basal segments elongated and rather slender; the internal flagellum consists of about twenty segments. The otocyst is oval.

The second antenna has a large scale, far exceeding in length the two joints of the peduncle (text-fig. 7).

The palp of the mandible may be four-jointed; it possesses a distinct exopoditic lobe, tipped with setæ, and the terminal segment is equal in length to the last but one (text-fig. 10).

The palp of the first maxilla is larger than in *Anaspides tasmaniæ*, and there are no setæ near it (text-fig. 13).

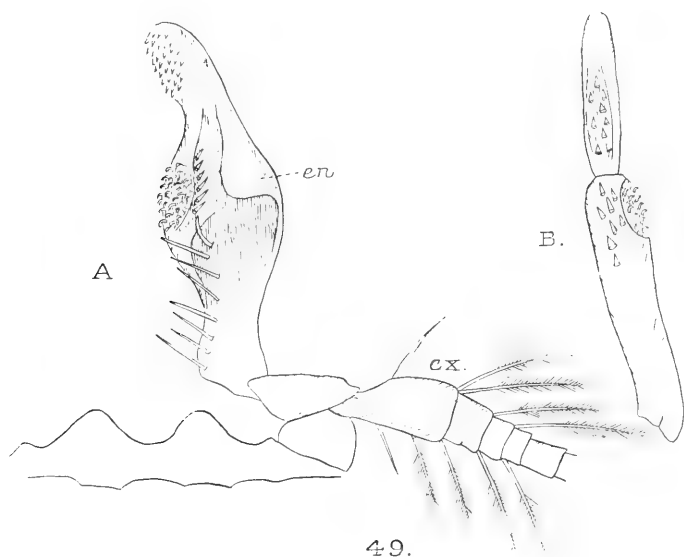
The second maxilla has a fairly well-developed exopoditic lobe fringed with long setæ (text-fig. 16).

The first thoracic appendage has two gnathobasic lobes, and the third segment of the limb is expanded inwards into a lobate biting blade (text-fig. 19).

The thoracic limbs are built upon a similar plan to those of Anaspides, but they are more slender and the setæ longer. The seventh thoracic limb has a small exopodite, but the segment bearing it is incompletely marked off from the other segments by a groove.

The first abdominal appendage of the male has a spatulate endopodite with an internal pad of recurved hooks, a

TEXT-FIG. 49.



Paranaspides lacustris, ♂. A. First abdominal limb. B. Second abdominal limb. *en.* Endopodite. *ex.* Exopodite.

proximal row of setæ, and the tip is furnished with a number of short spines (text-fig. 49A).

The endopodite of the second abdominal appendage of the male has an external pad of recurved hooks near the top of the first segment, and the terminal segment carries a number of stout spines (text-fig. 49B). There is no median spine on the sternum of this segment.

There is a row of short spine-like setæ on the posterior dorsal margin of the fourth, fifth and sixth segments.

The telson has an elongated form with slightly concave sides. The posterior margin is produced into a number of spines of very unequal length (text-fig. 33B and 34).

The uropods have a short basal segment; the exopodite has six or seven stout spines on its external border. The other setæ fringing the uropods are uniform.

The adult animal may attain an inch in length.

The colour is transparent green, but with a few minute black chromatophores scattered about, chiefly on the lateral portions of the segments.

Occurrence.—In the littoral zone of the Great Lake of Tasmania, among weeds and stones. Elevation 3700 ft.

Genus 3. *Præanaspides* (Woodward 13). (Text-fig. 3.)

The first thoracic segment is much shorter than the others; the succeeding thoracic segments are sub-equal in size and the abdominal segments are on the whole equal to the thoracic. There is no dorsal flexure. The antennal scale is apparently just equal in length to the first two joints of the endopodite. The segment of the thoracic limbs immediately proximal to the knee-joint is expanded especially in the anterior limbs. The sixth abdominal segment and the telson are together a little longer than the two preceding segments. The telson is equal in length to the uropods.

P. præcursor (Woodward 13).

The characters of this very important fossil are exhibited in text-fig. 3.

It is at once seen from these figures how close is the resemblance in all the essential characters between it and the living *Anaspides*. Similar transverse striations on the segments have been observed by Woodward in *Palæocaris*. With regard to the segmentation of the thoracic limbs it would appear that there were the typical three segments distal to the knee-joint and four segments proximal to it,

the separate segment bearing the exopodite having probably fused with the third segment, as in *Koonunga* and the posterior limbs of *Anaspides*. All the thoracic limbs except the last were apparently furnished with exopodites.

The abdominal appendages were probably very much as in *Anaspides*, the exopodites being furnished with long, slender hairs.

For the determination of specific characters probably the hind segments, telson and uropods are most important.

The dorsal posterior borders of the third, fourth, fifth and sixth abdominal segments have a moniliform ornamentation showing where a row of spines was situated. The telson is elongated and bluntly rounded at the end. Its lateral borders are setose almost to the base, and at the posterior lateral angles a few longer setæ were present.

The uropods do not project further than the end of the telson. The outer ramus has a row of about seven short setæ on its outer border and two elongated spines at the line of segmentation near the tips of this ramus.

Length.—Largest specimen 57 mm. in length.

Occurrence.—In clay-ironstone nodules of the Coal-measures near Ilkeston, Derbyshire.

Family II. *Koonungidæ* (Sayce 10 and 11).

The thorax is composed of seven distinct somites, the anterior one being fused with the head. The eyes are sessile. First antennæ of male with sensory modification. There is no antennal scale. The mandible has a cutting blade and a setose lobe, but no molar expansion. The palp of the first maxilla is reduced, but distinct, and carries setæ. The first thoracic limb has a slender exopodite, but is without gnathobasic lobes. The thoracic limbs are composed of only seven segments. The last two thoracic limbs are uniramous. The pleopods are all uniramous, except the first two pairs in the male, which are modified as copulatory organs and retain their endopodites.

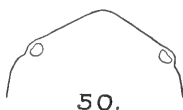
Genus 1. *Koonunga* (Sayce 10 and 11).

The cephalic segment is about equal in length to the two following segments: there is a short transverse sulcus on each side at about the middle distance, posteriorly to which the margins are produced downwards and inwards. The thoracic and abdominal segments are all subequal in size, the sixth abdominal segment not being longer than the segments preceding it. There is no dorsal flexure. The telson is very short and does not equal in length the uropods. The basal joint of the uropods is nearly as long as the rami.

K. cursor (Sayce 10 and 11). (Text-fig. 2.)

The frontal margin of the head is truncated and not produced into a projecting cone (text-fig. 50). There are no

TEXT-FIG. 50.



50.

Koonunga cursor. Anterior region of head with sessile eyes.

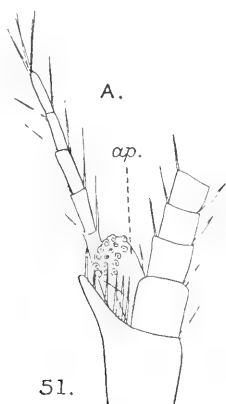
setæ upon it. The minute sessile eyes are situated at the angles of the frontal margin. The body is carried flat and unflexed. There is no median triangular piece marked out on the head. The head segment is as long as the two succeeding segments and there is a marked transverse sulcus on each side. The succeeding segments, both thoracic and abdominal, are all subequal. The first antennæ has the first segment distinctly stouter and longer than the two succeeding segments. The inner flagellum is six-jointed, and in the male a peculiar sensory appendage is present, furnished with spiral hairs (text-fig. 51). The otocyst is circular. The second antenna is without a scale (text-fig 8).

The mandible is without a molar lobe, and the terminal

segment of the palp is much less than half as long as the last segment but one (text-fig. 11).

The first maxilla has a large palp, tipped with three setæ. The lower biting lobe has only three setæ. The exopodite of the second maxilla is not marked by the presence of any setæ. The first thoracic appendage is much stouter than the

TEXT-FIG. 51.



Koonunga cursor, ♂. A. First antenna, showing sensory appendage (*ap.*). B. Spiral sensory hairs from appendage.

succeeding limbs; it is without gnathobasic lobes or expansions.

The succeeding limbs are very similar to those of *Anaspides* save that the upper series of gills are much smaller relatively, and the last two limbs are without exopodites.

All the thoracic limbs consist of seven, instead of eight, segments.

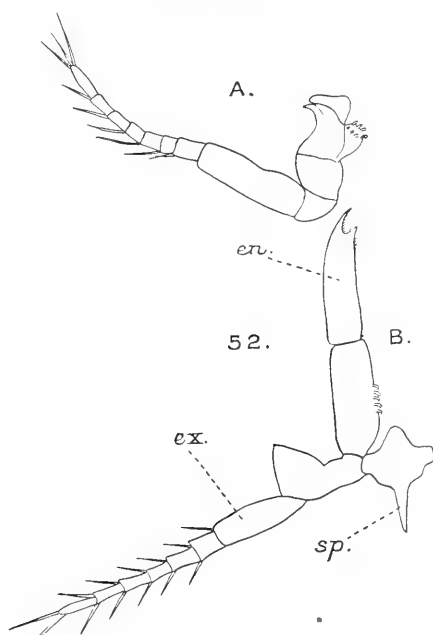
The exopodites consist of far fewer segments than in *Anaspididæ*.

The first abdominal appendage of the male possesses a broad endopodite which ends in a curved hook and a broad

blade; it is, in fact, definitely bifid. The pad of recurved hooks is borne on an internal projection (text-fig. 52A).

The endopodite of the second abdominal appendage of the male is two-jointed. The terminal joint is excavated at its tip and is clothed distally with very fine hairs. The pad of recurved hooks is situated internally on the proximal half of

TEXT-FIG. 52.



Koonunga cursor, ♂. A. First abdominal limb. B. Second abdominal limb. *en.* Endopodite. *ex.* Exopodite. *sp.* Spine on sternum.

the first segment. There is a large chitinous piece, with a posteriorly-directed spine, on the sternum belonging to this segment (text-fig. 52B). The posterior dorsal margins of the hind abdominal segments are without setæ or moniliform ornamentation. There is a single large spine on each side on the posterior dorsal margin of the sixth abdominal segment.

The telson is short and obtusely conical, and its posterior

and lateral margins are produced into a number of long stout spines of equal size. Between the spines are a number of short fine bristles (text-fig. 32).

The uropods have large basal segments furnished dorsally with three stout spines. The rami are short and truncated. The exopodite has about six long spines on its external border, and a number of long compound setæ terminally, which become shorter as we approach the internal border. The setæ clothing the endopodite are also much longer terminally than on the lateral borders.

The adult animal attains to about $\frac{1}{3}$ inch.

The colour is dark, marbled brown; ground colour yellow, with numerous black chromatophores.

Occurrence.—From freshwater reedy pools beside a tiny runnel joining the Mullum-Mullum Creek, Ringwood, near Melbourne.

Family III. Gampsonychidæ (Packard 5).

In this family may be included provisionally the three genera *Gampsonyx*, *Palæocaris*, and *Gasocaris*.

The thorax is composed of eight distinct segments of which the first is smaller than the rest. The succeeding segments, both thoracic and abdominal, are subequal in size, except the sixth which is somewhat elongated. An antennal scale was apparently present. All the thoracic limbs appear to have been biramous.

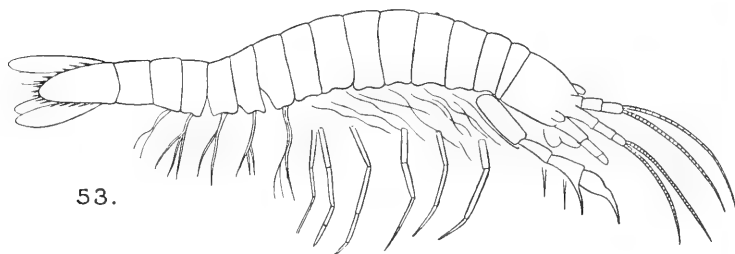
The body was carried straight without any flexure. The eyes were pedunculated.

Genus 1. *Gampsonyx* (Jordan and v. Meyer 1) (= *Gampsonychus* = *Uronectes*).

The flagella of the first antennæ were apparently equal in length. The first thoracic limb was a powerful raptorial organ armed with curved claws. The endopodites of the hinder thoracic limbs were slender and much elongated.

The telson was longer than the sixth abdominal segment. The abdominal appendages were apparently stout and flabellate.

TEXT-FIG. 53.

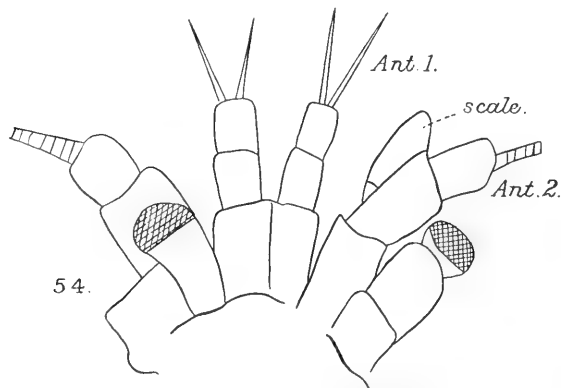


Gamponyx fimbriatus. Reconstruction after Jordan and v. Meyer.

G. fimbriatus (Jordan and v. Meyer 1). (Text-fig. 53.)

The structure of the antennæ and eyes is shown in text-fig. 54. The scale of the second antenna appears to have

TEXT-FIG. 54.



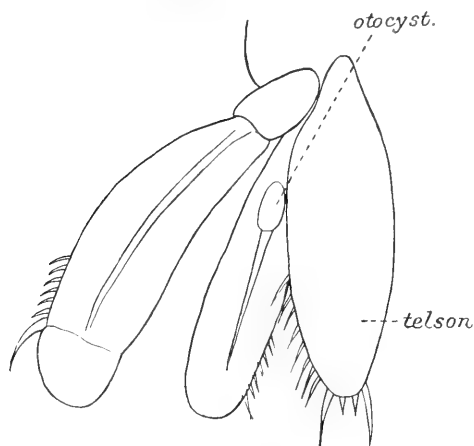
Gamponyx fimbriatus. Head; after Fritsch. *Ant. 1.* First antenna. *Ant. 2.* Second antenna with scale.

slightly exceeded in length the first segment of the peduncle. The posterior thoracic and the abdominal segments have well-marked pleura,

The first thoracic limb is a powerful raptorial organ of the structure shown in text-fig. 53. The next two limbs were rather small and fairly stout, but the succeeding five thoracic endopodites are very long and slender. Fritsch denies that any exopodites were present, but it may well be contended that the fine striæ figured by Jordan and v. Meyer springing from the bases of the thoracic limbs represent the hair-like setæ present on slender exopodites.

The abdominal appendages, according to Fritsch, are flabel-

TEXT-FIG. 55.



55.

Gamponyx fimbriatus. Telson and uropod. After Fritsch. late in structure, but Jordan and v. Meyer made them out to be setose.

Fritsch gives a very finely detailed drawing of the telson and uropods (text-fig. 55).

The telson was an elongated oval, with setæ on its posterior lateral margins, and two long and two short setæ at the hind end. The outer ramus of the uropod was about equal in length to the telson, and had a row of six short setæ and one enlarged spine on its outer border. Fritsch figures a conspicuous sphere on the inner ramus which he interprets as an otocyst.

Occurrence.—In the Carboniferous of Saarbrück, Rhenish Prussia, and of Lebach, Bohemia.

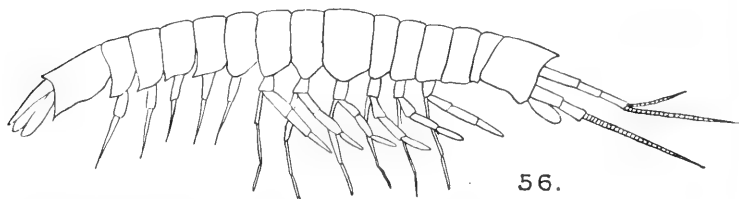
Genus 2. *Palæocaris* (Meek and Worthen 2 and 3).

The flagella of the first antennæ were unequal in size. The first thoracic limb was apparently not raptorial. The endopodites of the thoracic limbs were stout and short, exopodites elongated. The pleura of the abdominal segments projected backwards to end in a definite acute angle. The telson was shorter than the sixth abdominal segment. The abdominal appendages were slender, not flabellate.

P. typus (Meek and Worthen 2 and 3). (Text-figs. 56 and 57.)

The chief interest of this fossil is, perhaps, to be found in

TEXT-FIG. 56.



Palæocaris typus. Reconstruction after Packard. Lateral view.

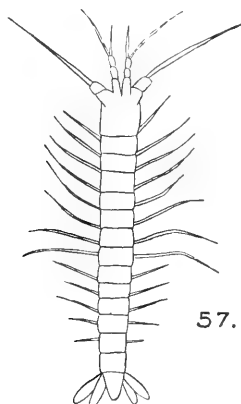
the fact that Packard definitely demonstrates the presence of exopodites on the thoracic limbs. The eyes are unknown, but since in so many respects this fossil is close to *Gamponyx* there can be little doubt that they were pedunculated. The thoracic limbs, if we can trust Packard's restoration, were all similar and biramous.

The telson was broad and short and apparently clothed with a uniform border of setæ. The uropods projected beyond the end of the telson and were also apparently clothed with uniform setæ.

In certain respects Meek and Worthen's figure (text-fig. 57) in dorsal view of *P. typus* is more interesting than

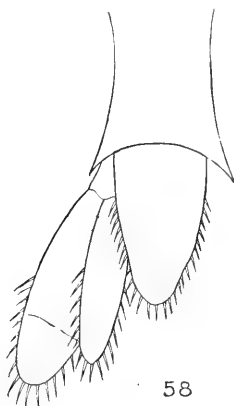
Packard's obviously diagrammatic restoration. The specimen is flat and resembles to an extraordinary degree the dorsal view of *Præanaspides* given by Woodward (text-fig. 3 B).

TEXT-FIG. 57.



Palæocaris typus. Reconstruction after Meek and Worthen.
Dorsal view.

TEXT-FIG. 58.



Palæocaris typus. Telson and uropod. After Packard.

We see also in this figure the limbs spread out laterally in the exact position assumed by *Anaspides* when walking.

Length.—.78 inch.

Occurrence.—In clay-ironstone concretions in lower Coal-measures of Mazon Creek, Morris, Illinois.

Woodward describes another species, *P. Burnettii*, measuring 30 mm. from the middle coal-measures of Irwell, Lancashire. In this species he describes the transverse striæ on the segments which he afterwards observed in *Præanaspides*.

Genus 3. *Gasocaris* (Fritsch 17).

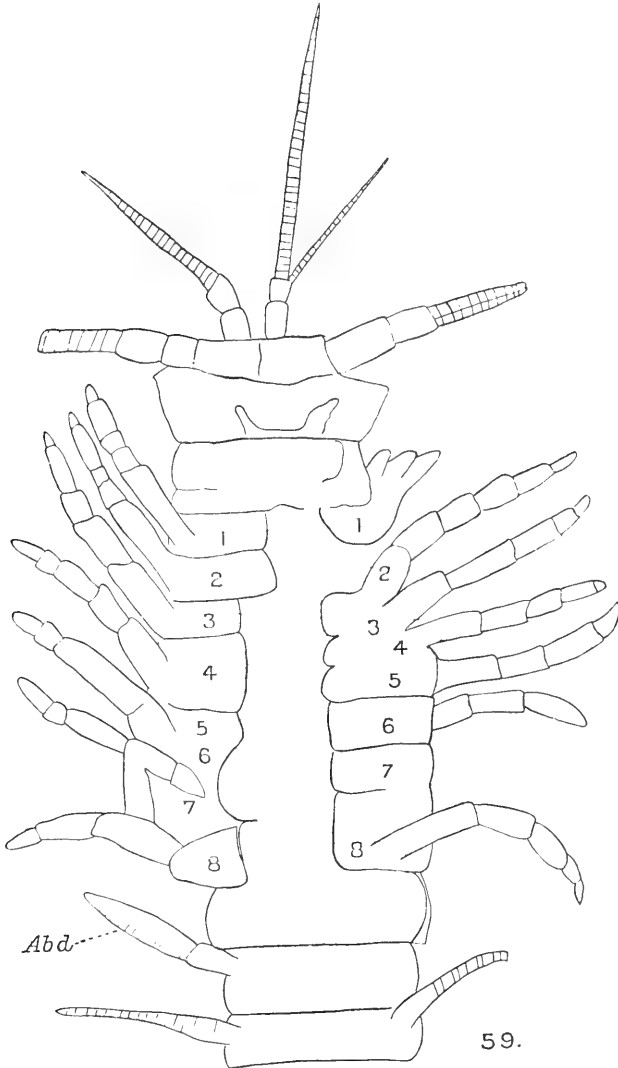
The flagella of the first antennæ are unequal in size. All the thoracic limbs are similar, rather short and stoutly built (without exopodites according to Fritsch). The telson is rather shorter than the uropods. There is a row of setæ on the posterior dorsal margin of all the thoracic and abdominal segments. The body is broad anteriorly, tapering considerably toward the hinder end. The abdominal limbs are stout, but annularly segmented.

G. krejci (Fritsch 17). (Text-figs. 59, 60, and 61.)

Fritsch establishes beyond doubt the pedunculated nature of the eyes and the structure of the first and second antennæ. In his restoration, however, he makes the two flagella of the first antennæ equal in size, which is contradicted by his figure of an actual specimen in ventral view (text-fig. 59). The scale of the second antenna seems to have reached the top of the peduncle on which the flagellum is inserted. With regard to the segmentation of the body his reconstruction is impossible to reconcile with the ventral view of an actual specimen. In the reconstruction he makes, besides a narrow head-segment, only six thoracic segments. In the ventral view, reproduced here, it is easy to make out eight free thoracic segments with limbs, and a broad segment in front to represent the head, and this structure would bring the specimen into line with the other *Anaspidacea*. The thoracic limbs, as reconstructed by Fritsch, are all similar, being rather short and

stout and apparently without exopodites, though with regard to this latter point we may well keep our judgment suspended,

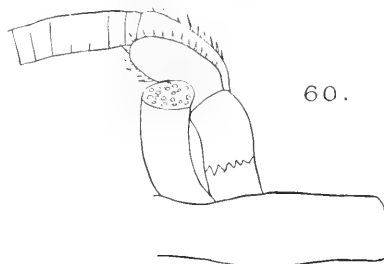
TEXT-FIG. 59.



Gasocaris krejci. Ventral view. Adapted from Fritsch. 1-8. Thoracic segments. *Abd.* Abdominal appendage.

owing to the difficulty of making out the exopodites in even the best preserved fossil Syncarida. The abdominal appendages

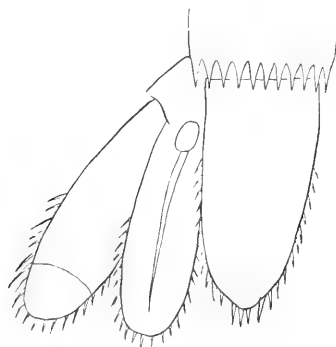
TEXT-FIG. 60.



Gasocaris krejci. Compound eye and second antenna. After Fritsch.

appear to have consisted of a stout outer ramus, segmented in the annular way characteristic of such a form as *Anaspides*. I cannot observe in Fritsch's figures of actual specimens any

TEXT-FIG. 61.



Gasocaris krejci. Telson and uropod. After Fritsch.

trace of endopodites, though Fritsch in his reconstruction figures the appendages as consisting of endopodite and exopodite of equal length. It appears more probable that the endopodite was the reduced flabellate structure which we know in the living *Anaspides*.

The telson is ovate or conical, with a border of short uniform setæ round the posterior three quarters of the margin. The uropods projected slightly beyond the telson and were fringed with uniform setæ. Fritsch figures an otocyst at the base of the inner ramus.

Length.—About 13 mm.

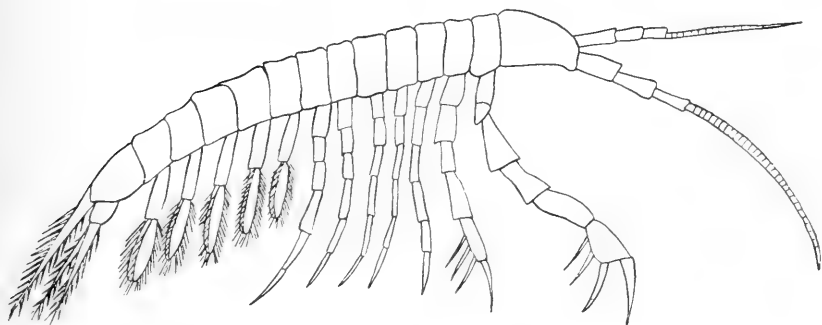
Occurrence.—In Coal-measures of Nyran near Pilsen; in older strata than those in which *Gampsonyx* occurs.

Genera of doubtful position.

Genus *Acanthotelson* (Meek and Worthen 2).

Two species are described, *A. stimpsoni* and *A. in-*

TEXT-FIG. 62.



62.

Acanthotelson stimpsoni. Reconstruction after Packard.
Lateral view.

æqualis. Packard gives a restoration of the genus. He figures besides a head segment, seven thoracic and six abdominal segments and a telson. The eyes are unknown. The first antennæ had a three-jointed peduncle and a single flagellum, according to the restoration, though it appears that it may have had two. The second antenna was apparently without scale. The seven thoracic limbs were long, fairly stout, and without exopodites. The first two limbs were raptorial in structure and furnished with long spines on the

penultimate joint. The abdominal appendages had a long basal segment and a terminal flabellate segment clothed with long setæ.

The telson was an elongated pointed spine fringed with long setæ. The uropods, which consisted of external and internal rami, were also long and pointed, and they were fringed with long setæ.

Occurrence.—Coal measures of Illinois.

Genus *Nectotelson* (Brocchi 19).

N. rochei from the Permian of Autun, France. Only very badly preserved specimens known.

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EXPLANATION OF PLATES 11 AND 12,

Illustrating Mr. Geoffrey Smith's memoir on "The Anaspidea, Living and Fossil."

PLATE 11.

FIG. 1.—*Anaspides tasmaniae*. Dorsal view of a very large specimen. Natural size, in normal position for running.

FIG. 2.—*Paranaspides lacustris*. Lateral view. Natural size, in position for running or swimming.

FIG. 3.—Shoot of the liver-wort, *Yungermannia*, with two eggs of *Anaspides tasmaniae*. $\times 5$.

PLATE 12.

FIG. 1.—Transverse section through otocyst of *Anaspides*, $\times 50$. *ec*, ectoderm; *n*, antennular nerve; *m*, muscle; *r*, setose ridge.

FIG. 2.—Portion of blood-forming organ of *Anaspides*, showing mitoses and detached corpuscles.

FIG. 3.—Transverse section through the cardiac portion of stomach of *Anaspides*. *C* and *D*, lateral ridges; *E*, dorsal median ridge; *H*, ventral ridge.

FIG. 4.—Transverse section through the alimentary canal of *Anaspides*, where the first diverticulum (*div.* 1) opens into the mid-gut. The section is not quite transverse, so that the opening is only seen on the right side. *B*, lateral chitinous ridge of pyloric division of the stomach extending into mid-gut; *b. m.*, basement membrane of mid-gut. *Div.* 1, first diverticulum, showing crowded nuclei and mitoses.

FIG. 5.—Transverse section through anterior portion of mid-gut, showing basement membrane (*b. m.*), and special gland-cells (*g.*).

FIG. 6.—Section through a special gland of mid-gut, showing flattened nuclei of gland-cells.

FIG. 7.—Section through wall of posterior part of mid-gut, showing absorptive layer (*m.*), basement membrane (*b. m.*), and submucosa (*s. m.*).

FIG. 8.—Transverse section through a liver tube of *Anaspides*, unfed condition. *g.*, special gland-cells.

FIG. 9.—Transverse section through a liver tube, full-fed condition with fat vacuoles in cells.

FIG. 10.—Transverse section through external duct of maxillary gland.

FIG. 11.—Section through a more internal part of gland.

FIG. 12.—Section through a more internal secretory part of gland.

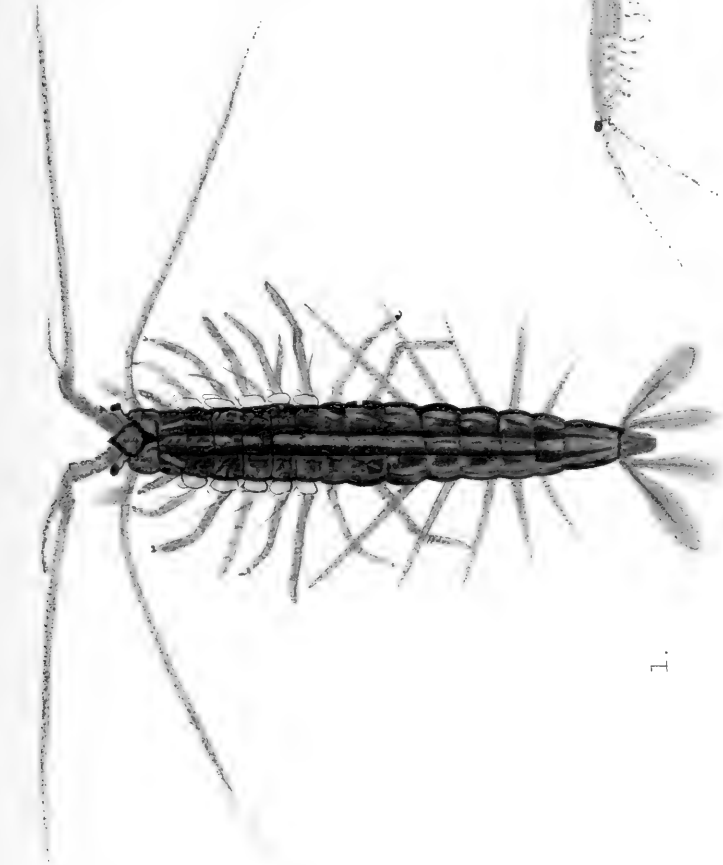
FIG. 13.—Section through a portion of end-sac.

FIG. 14.—Longitudinal section through a piece of ovary of *Anaspides*, showing two large ova, small ova lying in the lobes on the right, and the trophic cells (*tr.*) on the left.

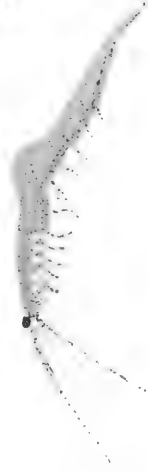
FIG. 15.—Transverse section through testis of young *Anaspides*, showing trophic cells (*tr.*), primary (*sp. 1*), and secondary spermatocytes (*sp. 2*), and spermatozoa (*spt.*).

FIG. 16.—Upper portion of vas deferens, with albuminous part of spermatophore in lumen, and trophic cells (*tr.*) surrounding it.

FIG. 17.—Section through the supporting tissue of glandular appearance which surrounds the ducts of the spermatheca, and is also present in the labrum.



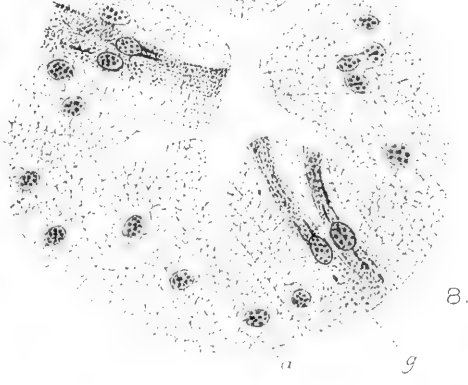
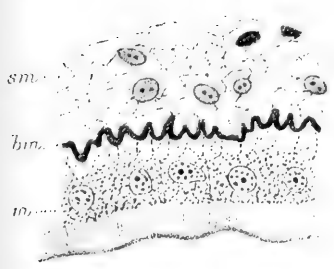
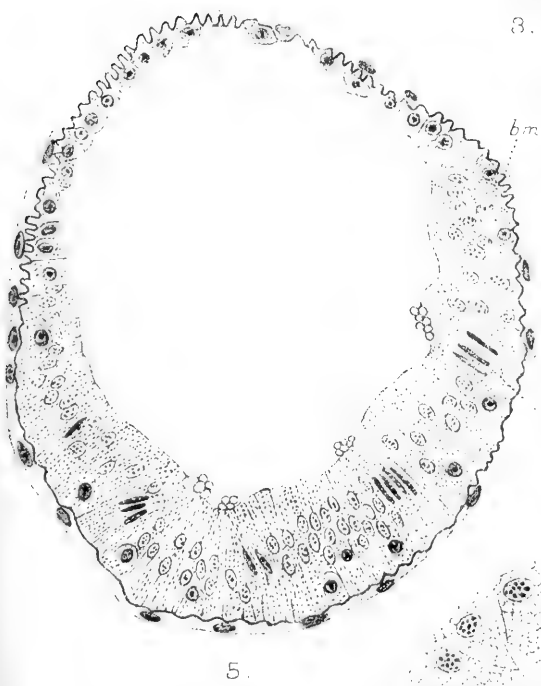
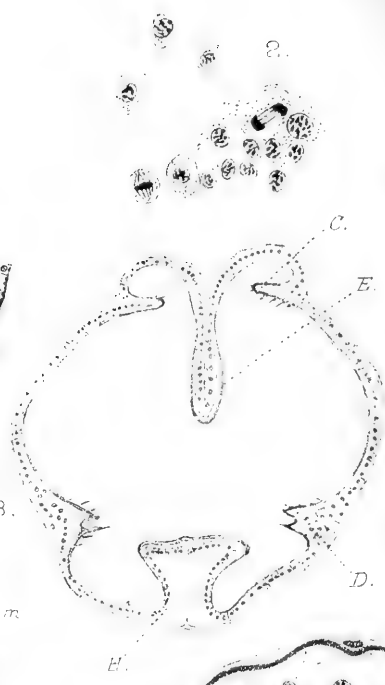
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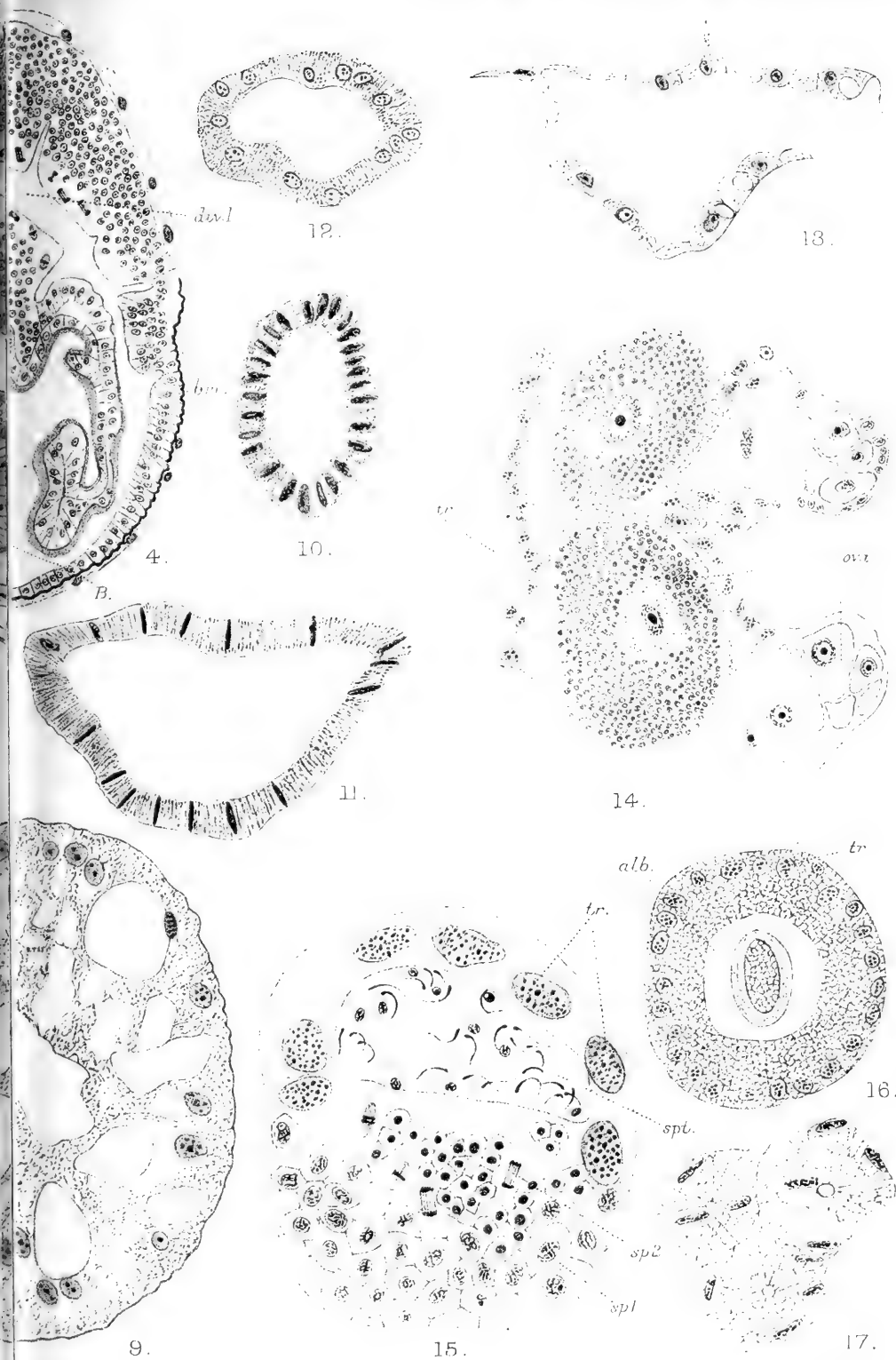


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On the so-called "Sexual" Method of Spore-formation in the Disporic Bacteria.¹

By

C. Clifford Dobell,

Fellow of Trinity College, Cambridge; Balfour Student in the University.

With Plate 13 and 3 Text-figures.

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INTRODUCTION.

Until the year 1902, no definite evidence regarding the sexuality of the Bacteria had been brought forward. It was generally supposed that the Bacteria constituted a group of very primitive organisms, in which the phenomenon of sex had not made its appearance. But with the publication of Schaudinn's remarkable researches on *Bacillus bütschlii*, it became apparent that such a supposition could not unreservedly be made. It appeared probable that—in *B. bütschlii* at least—a peculiar event in the life-history immediately preceding sporulation should be interpreted as

¹ I have already given a brief account of the work embodied in this paper to the Cambridge Philosophical Society, February 22nd, 1909.

representing a primitive or degenerate method of conjugation. If this interpretation is correct, then a fact of the utmost importance has been discovered—a fact of deep significance not merely as regards the affinities of the Bacteria, but also in relation to the general problem of sex.

For some years no confirmation of these observations was forthcoming; until, owing to the fortunate discovery of a new organism very like *B. bütschlii*, I was able—in 1907—to corroborate, in its essential points, the work of Schaudinn. Of the accuracy of Schaudinn's observations, I have myself no doubt whatever. But I cannot now regard his deductions from them as correct.

For various reasons, it seemed to me that the process which Schaudinn (1902) and others—including myself (1908)—were driven to regard as a form of conjugation, was, perhaps, capable of being interpreted in some other way. After much idle guessing, I concluded that the only method of throwing light upon the problem was to make a very careful study of sporulation in other Bacteria. Although so much has been written on this subject, it has been treated as a cytologic problem by but few investigators. And this is scarcely surprising, as the work has usually been done for medical purposes and upon organisms of very small size. One of the greatest difficulties which I encountered was that of obtaining species of Bacteria of sufficiently large size for exact observation. After many failures, I have now succeeded in following out the method of spore-formation in two large parasitic Bacteria which throw—as I believe—a considerable amount of light upon the “sexual” phenomena. It is my purpose in the following pages to describe these organisms and those stages in their life-histories which concern the problem under consideration. In a later part of the paper (p. 587) I shall point out the new point of view to which my researches have led me, and its consequences.

The descriptions which follow are based on a study of the organisms in their natural habitat. I have not isolated them

in pure culture, as my object has been to study their ordinary ways of life, and not those occurring under artificial conditions.

THE METHOD OF SPORE-FORMATION IN *BACILLUS SPIROGYRA*.

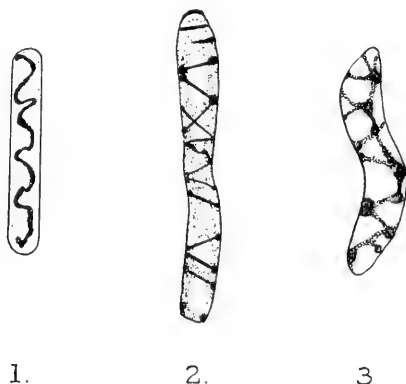
I have already (1908) given a brief description of the organism which I have named *Bacillus spirogyra*. I will here briefly recapitulate its main characteristics, and make a few additions and corrections to my original description.

B. spirogyra is a bacillus of large size—attaining a length of 12μ by ca. 2μ —which I have found in the large intestine of frogs and toads, chiefly the latter. It is very uncommon. The most striking characteristic of this *Bacillus* is a spiral filament which runs from end to end. This filament varies a good deal in the way it is disposed. Sometimes it appears almost straight (Pl. 13, fig. 1), though as a rule it is in the form of an irregular spiral or zig-zag (fig. 2). Occasionally the filament is very much contorted (fig. 3). It is always deeply stained by nuclear stains—especially good differentiation being obtained with Giemsa's stain and Heidenhain's iron-hæmatoxylin. From its constancy and staining properties I think it is justifiable to regard this structure as a nucleus. Owing to the refractivity of the organism's pellicle, the filament cannot be made out with certainty in the living cell. I have never found any granular or other inclusions in the cells.

Spiral filaments such as this have already been described in several Bacteria—notably by Swellengrebel in *Bacillus maximus buccalis* (1906) and in *Spirillum giganteum* (1907). They have also been figured frequently in spirochæts. Several recent investigators have considered that these filaments do not really exist as independent structures; they suppose them to be merely an appearance due to the arrangement of the granules and protoplasmic alveoli of the

cell [cf. Zettnow (1908), Hölling (1907), Guilliermond (1908), etc., and text-fig. A (3)]. That this is so in the case of *B. spirogyra* I cannot admit, nor, indeed, would anyone who had seen my preparations. I have already shown these to a number of persons, all of whom agree that such an interpretation is impossible. Not only are the varying disposition of the filament and its remarkable distinctness entirely against such an interpretation, but the appearance of burst forms (Pl. 13, figs. 4, 5) conclusively proves that the filament is a

TEXT-FIG. A.



B. spirogyra (1), *Sp. giganteum* (2, 3),—2, after Swellengrebel (1907); 3, after Guilliermond (1908). In *B. spirogyra* there is a simple filament. In *Sp. giganteum* there is (according to Swellengrebel), a kind of irregular rod-like spiral filament: according to Guilliermond the filament is falsely suggested by the arrangement of granules and cytoplasmic alveoli (3). The alveoli are, however, clearly shown by Swellengrebel (2) together with the filamentary structures.

solid body independent of the protoplasmic alveoli. In all carefully fixed and stained preparations the filament is as unmistakable as the nucleus of any other cell. In the case of *Sp. giganteum*, moreover, Swellengrebel has published figures which show both the filaments and the cytoplasmic alveoli in the same cell (see text-fig. A [2]).

I may add that I have been able to demonstrate the exist-

ence of a filament—independent of the cytoplasmic alveoli—in another *Spirillum*, which I hope to describe in a subsequent paper.

In my first account I stated that the filament in *B. spirogyra* became more twisted before division of the cell. This is not always the case; for I have found one or two individuals—though these are uncommon—which are dividing when the filament is in the form of an almost straight rod, (fig. 6).

Having said so much regarding the morphology of the organism, I will pass on to a description of the phenomena observed during spore-formation, which begins in the extreme posterior part of the host's rectum, but is not usually completed until the fæces have been discharged.

The first remarkable fact which I observed was that the individuals which were forming spores were little more than half the size of the ordinary individuals. After making measurements, I found that the average length of sporulating forms was about 5—6 μ , whilst that of ordinary individuals—selected at random—was roughly 9—10 μ . The reason for this was not difficult to discover. It appears that just before sporulation an ordinary transverse division of the cell takes place. Each daughter-cell then proceeds to form a spore without previously growing to the size of the ordinary individuals.

The details of spore-formation are as follows:—A large individual divides into two in the ordinary way—the chromatin filament being the first thing to divide (figs. 7, 8, 9). The daughter-cells produced by this division separate and undergo a certain amount of growth, though they never reach the size of the "parent" cell from which they were formed. Inside the cell the filament is seen to consist of comparatively few turns, and frequently displays a slight knob at one or both ends (fig. 10). One end of the filament now begins to enlarge—apparently at the expense of the rest of the spiral (figs. 11, 12)—so that a large nucleus-like mass is formed at one end of the cell (fig. 12). Up to this stage this body—

the spore rudiment—stains deep red, like chromatin, with Giemsa's stain. But a little later it changes its reaction, and is stained a bright blue (fig. 14). This, as I have already shown in *B. flexilis*, is owing to the formation of the spore membrane. As the membrane hardens the spore gradually stains less and less—until, finally, it refuses to stain at all (figs. 15, 16). At this stage the spore is fully formed, and the cell breaks up and liberates it more or less completely (fig. 16). It must be noted that a part of the filament persists outside the spore until quite a late stage, and breaks up with the rest of the cell (see figs. 14, 15, 16).

I occasionally found degenerating bacilli occurring together with those which appeared quite normal. Some of the forms encountered are shown in figs. 17, 18, and 19, and will require no further description.

A most remarkable—and, as I believe, important—variation in the method of forming spores was seen on a few occasions. The large original organism had failed to divide completely, and each of the small daughter individuals had developed spore rudiments whilst still attached to one another (fig. 13). [A discussion of these abnormal forms and their significance will be found on p. 587, et seq.]

THE METHOD OF SPORE-FORMATION IN *BACTERIUM LUNULA*, N. SP.

Under the name *Bacterium lunula* I will here describe another micro-organism which I have found in the rectum of toads. It is, like the preceding form, very uncommon and of considerable size. It reaches a length of about 15μ , though an average size is about 10μ — 12μ .

The chief characteristic of this organism is its curved shape (fig. 20). The ordinary individuals appear to have a chromatic filament similar to that of *B. spirogyra* (cf. fig. 20), but not so clearly defined. I cannot state definitely that

this is the normal condition, because on the few occasions on which I was fortunate enough to find these organisms, they were all beginning to sporulate. Hence, it is possible that the spiral arrangement of the chromatin appears only before spores are formed. I found very few individuals like that shown in fig. 20. Most of them had reached a more advanced stage in development.

The first stage in sporulation appears to be the constriction into two of a large form (fig. 21). The spiral is much more distinct at this stage. In some cases this constriction is so complete that two daughter-cells are formed, and separate as in *B. spirogyra*. Each daughter-cell, in a similar manner, then gives rise to a single spore (figs. 27, 28, 29). But in about an equal number of cases the constriction was incomplete or subsequently disappeared, and the large individual formed two terminal spores (figs. 22, 23, 25, 26), as in *B. flexilis*. The individuals which sporulate by the first method are therefore only about half the size of those which do so by the second method—these containing two spores, those one (cf. figs. 27 and 23). In the large individuals a trace of the original constriction was often to be seen (figs. 23, 26), and once or twice I saw forms in which it was still very pronounced (fig. 24).

Spore-formation appeared to take place just as in *B. flexilis* and *B. spirogyra*—that is to say, by an aggregation of chromatic material to form a spore-rudiment, and the subsequent formation of a spore membrane round this.

When the spore-rudiments are formed they resemble nuclei, and the organisms bear a very strong resemblance to a form recently described by Swellengrebel (1907 a) as *Bacterium binucleatum*. This bacterium contains two deeply-staining bodies, which careful micro-chemical tests lead Swellengrebel to suppose are nuclei. They appear to be constantly present, and divide in a curious manner. Another point of resemblance to *B. lunula* is the curved form which these organisms possess, thus causing them to resemble large *Vibrios*.

THE METHOD OF SPORE-FORMATION IN *B. BÜTSCHLI*,
B. FLEXILIS, AND OTHER DISPORIC BACTERIA.

In order that I may be able to discuss the general bearing of the foregoing observations on the central problem involved, I will here briefly summarise the descriptions which have been given of spore-formation in other disporic Bacteria.

First, I will recapitulate the process—the so-called “sexual” process—of spore-formation in *B. bütschlii* (Schaudinn, 1902) and *B. flexilis* (Dobell, 1908). The process is essentially the same in both (see text-fig. B, A, p. 589). A large individual (A 1), containing numerous chromatin granules, almost divides itself into two equal daughter-cells (A 2). The division is not completed, and subsequently all trace of it disappears (A 3). The granules now arrange themselves in the form of an irregular spiral, and at the same time begin to travel to opposite poles and mass themselves together to form the spore-rudiments (A 3, 4). Round these is formed a spore membrane, which gradually hardens and so gives rise to the completed resistant spore (A 5, 6). The remains of the cell, including a part of the chromatin spiral, perish.

Such is the process in these two Bacteria. If we search the literature bearing on the matter, we find that disporic forms are remarkably few—in many cases the existence of such forms is even denied, and it is stated that all Bacteria are monosporic. Several disporic Bacteria have, however, been observed; though in many cases it seems probable that the disporic individuals were exceptions, and monosporic individuals the rule. Prazmowski (1880) figured a large individual of a *Clostridium* sp. which possessed two terminal spores. Normally, however, but one spore is formed. In 1881 Kern described a large *Bacillus* from kephir, and proposed to name it *Dispora caucasica*, n. g., n. sp., on account of the fact that it regularly formed two terminal spores in each cell. De Bary (1884) in his text-book states that a single bacterial cell produces but a single spore:—

“Die seltene Ausnahme hiervon . . . dass nämlich zwei Sporen in einer Einzelzelle gebildet werden, kann in einem Uebersehen der Scheidewand zwischen zwei sporenbildenden Zellen ihren Grund haben.” This view has been held by the majority of other writers. I may mention, however, that Ernst (1888) described and figured certain cases in *Bacillus xerosis* in which two spore-rudiments were present, without any signs of a septum between them; and that Frenzel (1892) found unmistakable cases of the existence of two spores in his very large “grüner Kaulquappenbacillus.”¹ From the size of this organism, it seems to me unlikely that the septum—had it been present—would have been overlooked.

As far as I am aware, no other Bacteria which normally forms two spores have ever been described—and up to the present the process has been observed in detail in *B. bütschlii* and *B. flexilis* alone.

GENERAL CONCLUSIONS.

Schaudinn's interpretation of the phenomena observed in *B. bütschlii* was that the fusion of the two incompletely separated “daughter”-cells (text-fig. B, A 2, 3) represented a degenerate process of conjugation. A conjugation of this sort has been shown to take place in the yeasts (Schönning, Hoffmeister, Janssen and Leblanc, Guilliermond);² in the diatom *Achnanthes subsessilis* (Karsten);³ and in Protozoa, of which perhaps *Actinosphaerium* furnishes the best example (Hertwig).⁴ Conjugation of adjoining cells is

¹ This organism is of considerable interest in the present case, for it is another gigantic—occasionally disporic—form from *Anura* (tadpoles—probably of *Bufo marinus*). Frenzel describes the formation of the spore from a central nucleus-like body, but I think he was mistaken when he described the disporic forms as arising through the previous division of this body.

² See Barker, ‘Annals of Botany,’ vol. xv, 1901.

³ See Klebahn, ‘Arch. Protistenk,’ Bd. 1, 1902.

⁴ R. Hertwig, ‘Abh. Akad. München,’ xxix, 1898.

also known to occur in some filamentous Algæ—in *Sphæroszoma* and *Spondylosium* (Desmidiaceæ),¹ and in *Spirogyra*, *Zygnema*, and other *Conjugatæ*. A comparable process has also been described in the Metazoa—in the parthenogenetic egg of *Artemia*.²

Now it appears to me, since observing the sporulation of *B. spirogyra* and *B. lunula*, that a much simpler interpretation of these phenomena in Bacteria is possible: namely, that we see here not a degenerate sexual process, but merely an abortive cell division. We have seen that a division takes place in *B. spirogyra* just before spore-formation, and that the daughter-cells which are so formed proceed to form spores without growing to their full size. We have only to suppose that this last cell division is not completed, to arrive at a result such as we see in *B. bütschlii*. There is, indeed, evidence to show that this last division may abort in *B. spirogyra* (cf. Pl. 13, fig. 13), so that individuals like the sporulating individuals of *B. bütschlii* result. My meaning will be made clearer by a glance at the accompanying figure (text-fig. B).

I mean to say that the disporic individuals which we find in *B. bütschlii* and *B. flexilis* are really double individuals—two individuals of the last generation of the life-cycle which have not been completely separated. It is a cell-division, not a sexual act, which has regressed. In *Bacterium lunula*, I believe, the regression has not gone so far, so that the last cell-division may be complete—giving rise, therefore, to the monosporic individuals—or abortive,—giving rise to the disporic individuals, as in *B. bütschlii*.

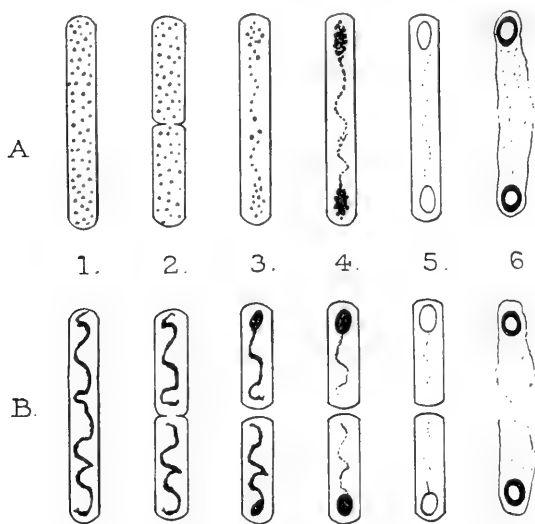
Schaudinn described abnormal monosporic individuals in *B. bütschlii*, and I also found them in *B. flexilis*. After I had reached the conclusions given above, I re-examined my old preparations of *B. flexilis*, and found several interesting abnormalities. In the first place, I found that the mono-

¹ De Bary, "Untersuchungen über die Familie der Conjugaten," Leipzig, 1858.

² Brauer, 'Arch. mik. Anat.,' 1893.

sporic individuals were nearly always very much smaller than those with two spores, but looked quite normal in other ways (figs. 31 and 32 are two instances). And I also found—though these were rare—forms which had remained almost completely divided right up to a late stage in sporulation (fig. 30). I thus felt that my original deductions from B.

TEXT-FIG. B.



A. Spore-formation in *Bacillus bütschlii* or *B. flexilis*.
B. Spore-formation in *B. spirogyra*. (Diagrammatic.)

spirogyra and *B. lunula* were considerably strengthened. There is absolutely no evidence whatsoever that any process of conjugation takes place in *B. spirogyra* before the final cell division. Nor do I think any sexual significance would have been attributed to the phenomena in *B. bütschlii* if the sporulation of *B. spirogyra* had been previously known. The comparison with yeasts and algæ is, I believe, wholly misleading. I would also point out that the process in *Actinosphærium* is not in any case strictly comparable with that in *B. bütschlii*: for in this—assuming conjuga-

tion occurs—two zygotes result, whereas in that only one is formed.

The streaming of the granules observed so carefully by Schaudinn in *B. bütschlii* is due, I believe, merely to the fact that they have to travel to the poles of the cell to form the nucleus-like spore-rudiments.

Why the last division should abort in the disporic forms, must remain an open question. A possible explanation is that it is a process correlated with the parasitic manner of life. When the organisms are removed from their host in the

TEXT-FIG. C.



Bacilli (sp. incert.) from rectum of *Bufo vulgaris*; sporulating. x = a double individual. [Sublimate-alcohol, iron-alum hæmatoxylin; 2 mm. apochrom. x comp.-oc. 18. ($\times 2500$).]

fæces, they would soon—in all probability—be dried, and hence induced to enter the resting state. And for purposes of dissemination, it is obvious that two spores are always better than one, so that the last division—albeit abortive—would double the number of individuals capable of sporulating.

In my preparations of *B. spirogyra* were many other Bacteria,—amongst them a small *Bacillus* which was also sporulating. The ordinary individuals formed a single terminal spore (text-fig. C) but here and there, double forms were to be found (x), which had spores placed at opposite poles. I find that similar forms have often been figured: for instance, Guilliermond (1908) gives several good examples

(pl. 3, figs. 36—39, etc). In such organisms—which are obviously two still-attached individuals—the terminal position of the spores is remarkable when considered in relation to the disporic forms. Just as the latter have failed to form a complete septum, so have the former failed to separate after forming the septum.

The significance of the spiral configuration of the chromatin at a certain point in spore-formation in *B. bütschlii* and *B. flexilis* (text-fig. B, A 3, 4) still remains obscure. That it is connected with “conjugation” can scarcely be maintained, however, for as we have seen, it is permanently present in *B. spirogyra*. Perhaps a study of spore-formation in other forms may serve to throw some light upon its meaning. It may be noted, moreover, that Mencl (1905) has found similar arrangements of the chromatin at certain periods in the life-history of some of the remarkable filamentous forms which he studied.

It seems to me that the parallel between the method of spore-formation in *B. spirogyra* and that of *B. bütschlii* is too close to be merely accidental. We have seen that the abnormal forms of the one are the same as the normal forms of the other. And when we consider further that in another case (*B. lunula*) spore-formation proceeds sometimes as in *B. spirogyra*, sometimes as in *B. bütschlii*, then it appears to me certain that the process of sporulation is really essentially the same in all these forms. If this is so, then there is but one alternative to my interpretation given above. It is, that conjugation takes place in all these forms, but that in *B. spirogyra* (always) and in *B. lunula* (sometimes) the conjugants separate before forming spores. As I have already said, there is absolutely no justification for such an assumption. The nuclear filament undergoes no changes which could be so interpreted—either in *B. spirogyra* or *B. lunula*. The significance of the “conjugation” of *B. bütschlii* and *B. spirogyra* is, to me, now quite clear: the last transverse division in the life-history has, for some reason, become abortive—division begins, progresses for a

short way, and then regresses. But in this process two individuals are formed—each capable of sporulating—so that division is really completed physiologically, though not morphologically.

Now if my interpretation be correct—as I believe it is—it has some interesting results. In the first place, it has an important bearing upon the problem of the affinities of the Bacteria. Although there can, I think, be little doubt that the Bacteria are a very heterogeneous group, yet they present—as a whole—some well-defined features. And if we consider these, it seems to me that their affinities are not with the yeasts, as is often supposed, but in part with the Algæ and in part with other organisms. All recent work appears to me to show that the yeasts are really a low group of Fungi, which is properly placed in or near the Ascomycetes.

The similarity between the “conjugation” of the disporic Bacteria and the conjugation of yeasts furnished a strong piece of evidence in favour of the close kinship of these two groups—a piece of evidence which was, to me, wholly puzzling, but which is now, I think, shown to be false. My observations on *B. spirogyra* refute, I think, one of the strongest arguments in favour of the affinities of the Bacteria with the yeasts.¹

There is one other point I must mention before I conclude, and that is with regard to the general phenomenon of sex. As is well known, Schaudinn—in his last papers (cf. Schaudinn, 1905)—advanced the view that sexuality is a fundamental property of living matter, having arisen when life itself arose—“so halte ich die Befruchtung für einen allen Lebewesen zukommenden Vorgang” (p. 34). This idea has been taken up and extended by Prowazek (1907), who has sought to show that sexuality is universal in the Protista, and that it is in some way correlated with the “diphasic nature” of protoplasm. How “sol”-phases and “gel”-phases of colloidal substances are connected (outside

¹ The name “Schizomycetes” is, I think, quite misleading when applied to the Bacteria, and should be dropped.

the imagination) with maleness and femaleness is—to me—quite a mystery. That there may be a connection, I cannot deny; but at present it seems to me that there is no more justification for such a view than there would be for correlating males and females with gas-engines and steam-engines.

I think Schaudinn's view was largely influenced by the fact that he found sexual phenomena not only in many Protozoa, but also—as he believed—in the Bacteria. If, then, it can be shown that the “sexual” process does not occur in Bacteria, one of the chief supports of the view vanishes. And—as I believe—the observations recorded in the beginning of this paper show that—in the disporic forms at least—no real sexual process occurs. For my own part, I do not think the evidence supports the hypothesis that sexuality is a fundamental property of living matter. And certainly I see no evidence in favour of such a hypothesis derived from the Bacteria.

I confess there is still one unaccountable case of “sexuality” described in Bacteria—that of *B. sporonema* (Schaudinn, 1903). This organism, however, stands alone. Perhaps future work will give us an explanation of the meaning of its “conjugation.” At all events, it is premature to generalise from this single case at present. I hope my own researches—which are still in progress—on the Bacteria and allied forms may shed some further light upon this most interesting phenomenon.

ZOOLOGICAL LABORATORY,

CAMBRIDGE;

February, 1909.

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DESCRIPTION OF PLATE 13,

Illustrating Mr. C. Clifford Dobell's paper "On the so-called 'Sexual' Method of Spore-formation in the Disporic Bacteria."

[All figures are from preparations fixed with formalin and stained by a modification of Giemsa's method. Drawings made under a Zeiss 2 mm. homog. oil immersion apochromatic, comp.-oc. 12. Magnification ca. 2000 diameters.]

Figs. 1—19. *Bacillus spirogyra*.

Fig. 1.—An individual with an almost straight nuclear filament.

Fig. 2.—An individual with well-marked spiral or zig-zag filament.

Fig. 3.—An individual in which the filament is very greatly contorted.

Figs. 4, 5.—Two burst individuals, in which the filament is clearly seen to be a solid independent structure lying in the cell.

Fig. 6.—Division of an individual with a straight filament.

Figs. 7—16. Stages in spore-formation.

Figs. 7, 8, 9.—Stages in division of a large individual into two.

Fig. 10.—Small individual formed by division of a large one—about to form a spore.

Fig. 11.—A similar organism at a later stage. One end of the filament is much enlarged, forming the spore-rudiment.

Fig. 12.—A later stage, in which the spore-rudiment is further developed.

Fig. 13.—An abnormal case, in which a large individual has not completely divided, and in which each of the (still attached) daughter-individuals contains a spore-rudiment similar to Fig. 11.

Fig. 14.—Stage succeeding Fig. 12. The spore-membrane has just been formed, and is stained a bright blue.

Figs. 15, 16.—Later stages—completion of spore-formation. In Fig. 16 the spore is fully formed, and the remains of the cell are breaking up.

Figs. 17, 18, 19.—Degenerate forms.

Figs. 20—29. *Bacterium lunula*, n.sp.

Fig. 20.—Ordinary individual before sporulation.

Fig. 21.—Individual dividing into two, preparatory to forming spores.

Figs. 22, 23, 25, 26.—Successive stages in the development of a disporic (double) individual.

Fig. 24.—An individual, almost divided into two—each half containing a spore-rudiment.

Figs. 27, 28, 29.—Stages in development of a spore in a monosporic individual.

Figs. 30—32. *Bacillus flexilis*.

Fig. 30.—An abnormal individual, which—at a late stage in spore-formation—is almost completely divided into two.

Figs. 31, 32.—Stages in development of (small) abnormal monosporic individuals.



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31.



32.

Studies on Polychæt Larvæ.

By

F. H. Gravely, M.Sc.,

Junior Demonstrator of Zoology in the Victoria University
of Manchester.

With Plate 14 and 3 Text-figures.

DURING July, 1907, whilst studying in a general way the pelagic larvæ of Port Erin, my attention was specially turned to the Polychæt larvæ on account of their abundance and the difficulty I experienced in identifying them. As a result of this I spent part of the following summer in a more careful examination of these larvæ. I am publishing a systematic account of the results of this investigation elsewhere (Gravely, 1909). In the present paper I propose to describe a young specimen of a pelagic species of *Odontosyllis*, and the "vestibule" and associated modifications of the anterior end of larvæ of the *Spionidæ* and *Polydoridæ*; and in conclusion I shall discuss certain other points of general interest arising from the above investigations. As, however, there appears to be no account in the English language of the terminology commonly adopted in the description of polychæt larvæ, it will probably serve a useful purpose if the first part of the paper is devoted to this subject.

TERMINOLOGY.

Prof. Häcker (1897, pp. 74-76) distinguishes the following larval stages :

(1) *Protrochophore*: An unsegmented larva in which the "preoral" ciliated band is represented by an extremely broad band of short cilia (text-fig. 1).

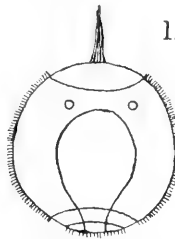
(2) *Trochophore*: This differs from the last only in having the diffuse ciliated band replaced by a narrow band of long cilia.

(3) *Metatrochophore*: The simplest form of segmented larva:

(a) In the first metatrochophore stage no parapodia are present.

(b) In the second metatrochophore stage parapodia appear, but do not function as organs of locomotion.

TEXT-FIG. 1.



Protrochophore of a Eunicid (from Häcker, after Claparède and Mecznirow).

(4) *Nectochaeta*: The preoral ciliated band is replaced as primary swimming apparatus by the parapodia with their setæ. This is the definition given by Prof. Häcker, but it is usually extremely difficult to determine the period at which this change takes place. Even in the case of *Polynoë*, in which the larva continues its pelagic existence for a considerable time after the disappearance of the ciliated band, the more advanced pelagic larvæ settle to the bottom when in captivity and use their parapodia for crawling only, those specimens in which the ciliated band is still present holding the parapodia motionless until movement by means of the cilia is impeded. The term has, therefore, come to be used some-

what arbitrarily, and I have used it to denote a morphological rather than a physiological stage of development.

(5) The larva usually takes to its life on the sea bottom at the close of the nectochæta stage, but in the case of *Polynoë* at least the nectochæta stage is commonly regarded as ending after a period of partially arrested development, although the pelagic life is continued beyond this until several additional segments have been developed.

In some Polychæts the metatrochophore is succeeded by an elongated larva in which swimming is effected by means of undulatory movements of the body; this form may be distinguished from the Nectochæta under the name Nectosoma.

A larva is said to be monotrochal when only the "preoral" ciliated band is present, and telotrochal when it bears in addition the "perianal" band.

In larvæ of the Chætopteridæ the ciliated band is situated in a position which ultimately becomes separated from the head by several segments—such a larva is said to be mesotrochal.

A segmented larva that bears several ciliated bands at intervals along its body is said to be polytrochal.

A larva bearing no ciliated band is often referred to as an atrochal larva, though this term is not given in Prof. Häcker's list. It should be pointed out, however, that this term has often been used to describe larvæ in which an extensive tract of short cilia encircles the body (as in the protrochophore and immediately succeeding stages of Eunicid larvæ); such a larva is not atrochal in the restricted sense in which the term is used in the present paper.

The "preoral" ciliated band may be termed the prototroch to distinguish it from any band situated between itself and the apical plate, such a band being called an akrotroch. Any band posterior to the prototroch is spoken of as a paratroch, and of these the "perianal" band, situated upon the terminal segment (posterior to the formative region of the body), may be distinguished as the telotroch (Lankester; = "Endparatroch," Häcker), from the remaining inter-

paratrochs ("Zwischenparatrochen," Häcker). The band of a mesotrochal larva is called a mesotroch.

The extension of the preoral lobe that often occurs in the plane of the prototroch in order to increase the extent of this important swimming organ is called the umbrella.

In the Nereidiformia and some other worms there usually develop during the metatrochophore stage a number of segments, definite for each species, all of which appear at about the same time and whose parapodia become very fully developed before any further segments are added; the former (including the peristomial, but excluding the anal) are called primary, the latter secondary, segments.

To this list of Prof. Häcker's terms may be added the following, derived from the names of the three groups into which Claparède divided his class "Metachætæ" (Polychæt larvæ with provisional setae, see Claparède 1863, p. 87); gastrotroch, a ciliated band extending transversely across the ventral surface of a segment; nototroch, a similar dorsal band; and amphitroch, a complete girdle of cilia.

Finally, I have found it convenient to use the term neurotroch for the tract of short cilia that often extends upon the ventral surface of the larva along the course of the nerve-cord.

DESCRIPTION OF AN INTERESTING YOUNG SPECIMEN OF ODONTOSYLLIS SP.

When towing a weighted net between Aldrick Bay (S. of Port Erin) and Gibdale Bay (Calf of Man), I had the good fortune to obtain a single young specimen of the brown *Odontosyllis*, frequently seen in the adult condition—occasionally accompanied by *O. ctenostoma*, and sexual specimens of *Autolytus* and *Myrianida*—swimming at the surface of the sea at the mouth of Port Erin Bay and further out towards the Calf on calm evenings during July. The genus *Odontosyllis* as defined by Langerhans (1879, p. 525) is characterised by the presence of distinct palps and of a

series of ventral denticulations at the anterior end of the pharynx, but no strong tooth or similar dorsal denticulations. The present species is closely allied to *O. gibba* (Claparède, 1863, pp. 81-83; Pl. XII, figs. 7-14). It differs therefrom, however, in that special natatory setæ are present on the parapodia of the eighth or ninth and succeeding segments in both sexes, instead of from the seventh in the male, and from a segment further back (eleventh?) in the female (De Saint-Joseph, 1886, p. 174); these setæ, moreover, would appear to be present throughout life in the present species (see below), instead of at maturity only as in *O. gibba*. De Saint-Joseph also notes a single capillary seta in each ventral bunch of the posterior segments of his specimens of *O. gibba*, but I have been unable to discover this in mature specimens of the present form, although in the young specimen it occurs in every tuft throughout the whole length of the body. Lastly, in this form it is the peristomial, not the first chætigerous, segment that is produced forwards dorsally in contracted specimens at least, so as to overlap the posterior part of the head.

The jointed setæ closely resemble those of *O. gibba* (Claparède, 1863, Pl. XII, fig. 7A), though their proximal segment terminates in a somewhat sharper point (text-fig. 2), and the shortness of the tentacles and cirri and the presence of six denticles is quite characteristic of *O. gibba*. I have been unable up to the present to find any description of a species differing from it only in the above-mentioned points.

Only one specimen of this worm was obtained in a really young condition, and I was unable to examine this alive. It was quite small, being only 1.75 mm. long, and possessing but twenty-two segments, each about 0.6 mm. broad (exclusive of appendages). The adult worm may have as many as fifty segments, 2 mm. in breadth, the whole being 15 mm. long.

The anterior end of the young specimen is rounded, but consists in reality of a pair of large, closely apposed palps, projecting in front of the transversely flattened head;

in proportion to the size of the head they are very much larger than in the mature form. Lateral cephalic tentacles are present as short projections from the head, but I have been unable to distinguish the median tentacle. It is impossible to determine with certainty from the mounted specimen whether any ciliated bands are present or not.

The peristomial segment is extremely short, and apparently bears no cirri at this stage. The next seven segments bear uniramous parapodia. These are probably all provided with

TEXT-FIG. 2.



Compound seta of pelagic *Odontosyllis*; from a mature specimen.

dorsal cirri, though those of the first two pairs are very difficult to distinguish. Each of these uniramous parapodia is supported, as in the adult, by a single stout aciculum which is straight, or nearly so; and each bears a tuft of setæ, one capillary, and the rest compound with the comparatively long distal segment characteristic of *O. gibba*.

The parapodia of the next twelve segments show in addition a broad dorsal ramus, supported by a shorter and more distinctly curved aciculum than is present in the longer ventral ramus, which ventral ramus resembles the only ramus of the anterior parapodia. The dorsal ramus bears a tuft of very long slender capillary setæ, exactly like the special natatory setæ that are found in this position in sexually mature specimens of this and many other Syllids. Finally, at the posterior end of the body there are two chætigerous segments devoid of the dorsal lobe and natatory setæ, and evidently not yet fully developed, the tissues taking a deeper stain, and the tuft of compound setæ being shorter (especially in the terminal one) than in the segments preceding them.

The gut is fully differentiated, and divided into pharynx—in which, however, I have been unable to detect any teeth, although De Saint-Joseph figures all six of them in a much younger (five-segment) larva which he refers to *Odontosyllis gibba* (1886, Pl. VIII, fig. 40)—gizzard, and intestine, the last being deeply constricted between the segments.

The body is covered externally with minute particles of some highly refractive foreign substance—possibly caught simply by the mucus that these worms not infrequently exude under the action of the fixing agent.

It will be seen from this description that we have here a Syllid in which true biramous appendages appear at a very early age. These are strictly comparable to those found in the mature form, occurring on precisely the same segments so far as the development of these permits, and showing precisely the same structure. It seems reasonable to suppose, therefore, that they are present throughout life, and from this it may be concluded that the species is permanently pelagic. So far as I know it has never been dredged up from the bottom, but so little work has as yet been done upon the Polychæt fauna of the Port Erin district that the only definite information to be had in regard to this species is that it is to be found swimming at the surface on calm

summer evenings. The young specimen was, it is true, taken in a weighted tow-net sunk on a long line in water whose depth was probably about 10 fathoms, but the presence in the net of the earlier stages only of the *Pluteus* of *Echinocardium cordatum* shows that the material amongst which this specimen was found was brought up from a level some distance above the bottom; also, as our object was simply the collection of material and not a comparison between surface and deep-water plankton, no precaution was taken to prevent specimens from getting into the net on its way through the surface waters, so this little worm may in reality be a surface form.

Many Syllids, including those of the genus *Odontosyllis*, develop special natatory setæ and take to a pelagic existence at the time of sexual maturity. In these cases each tuft of natatory setæ springs from a broad muscular dorsal lobe of the parapodium which is supported by several stronger setæ, or by a definite aciculum, and the parapodia bearing them usually show a more or less definite relation to the segments in which the sexual cells are produced, occurring only either upon or (as in the male *Autolytus*—"Polybostrichus") behind them. It has been pointed out by Malaquin (1891, also 1893, pp. 427-430) that when special natatory setæ appear at the time of sexual maturity on appendages of those Syllidæ which normally bear a ventral cirrus, then these appendages show the complete normal structure of a *Polychaet* parapodium; and he has further pointed out that the essential parts are always developed in the following order—ventral ramus, dorsal cirrus, ventral cirrus, dorsal ramus, and that when any of these are suppressed it is always in the reverse order. The only exception to this rule that he finds is that forms devoid of ventral cirrus before maturity may develop the dorsal ramus without it.

It would appear from his account, too, that in no known case is the dorsal ramus developed until the time of sexual maturity. It seems, therefore, that the primitive Syllid stock was characterised by the loss of the dorsal ramus.

Further, this ramus, when developed in connection with the reproductive processes, appeared in close association with the sexual cells, both in regard to the time of its appearance and to the position of the segments which came to bear it. In the present species of *Odontosyllis*, the period of pelagic existence has apparently been extended from the time of sexual maturity till it has become life-long; and in connection with this, the time of appearance of the special natatory portions of the parapodia has been thrown back to quite an early stage in the development of the worm. The biramous parapodia are, moreover, very fully developed, the dorsal ramus being quite separate from the ventral one distally, and supported by a single stout aciculum.

The fact that the appendages with a dorsal ramus occur on the gonad-bearing segments, and on not more than one segment anterior to these, shows them to be strictly comparable to the biramous parapodia developed by those *Syllids* which lead a pelagic life at the period of sexual maturity; they are, therefore, only primitive in that they have reverted to the fundamental biramous type of Annelid appendage. They have not been directly derived from similar appendages of the primitive Polychæt stock. We have here, therefore, an example of the precocious development of an organ in connection with the precocious assumption of a particular mode of life.

The ventral cirrus is apparently not present even in the adult of this worm; certain parapodia may often be seen to bear a conspicuous cirrus-like outgrowth of the ventral ramus, but in others this is shorter and proves to be simply a prolongation of one of the three lobes surrounding the base of the tuft of setæ.

Claparède (1863, Pl. XII, fig. 12) and De Saint-Joseph (1886, Pl. VIII, fig. 40) both describe larvæ of *O. gibba* in a much earlier stage than the young *Odontosyllis* described above. These are polytrochal, and appear to show no trace of the dorsal lobe of the parapodium. In De Saint-Joseph's larva, however, only six segments are present, and in

Claparède's eleven-segment larva the posterior segments are perhaps insufficiently developed to show the dorsal lobe in any case.

McIntosh (1908, pp. 112-3, text-fig. 45) briefly describes a minute pelagic worm, about one eighth of an inch long, in which "every foot from the second to the last is furnished with a very long translucent tuft of swimming-bristles." Only a single specimen of this worm is known, and its precise position is uncertain, but McIntosh states that it seems to be most nearly related to the Syllidæ and Alciopidæ.

EXTERNAL FEATURES OF THE HEAD AND ANTERIOR SEGMENTS IN LARVÆ OF THE SPIONIDÆ AND POLYDORIDÆ.

In the Nectosoma larvæ of the Spionidæ and Polydoridæ there is a capacious "mouth" bordered by a pair of large lateral lips. These structures appear to be quite characteristic of the two families, and to have caused in them considerable modifications of the ciliation of the anterior segments. They have been briefly referred to by Claparède and are indicated in his figures of the larva of *Polydora* Bosc., (= *Leucodora*, Johnst.) and in his figures of Spionid larvæ (1863, Pl. VI, figs. 1 and 3; Pl. VII, figs. 1, 6, and 7), and in Cunningham and Ramage's figure (1887, Pl. XXXVII, fig. 2J) of an advanced larva referred by them to *Nerine cirratulus* they are very conspicuous. No full account of them has as yet been published, however, and many authors appear to have overlooked or ignored them altogether, in spite of their great importance in the morphology of these larvæ.

In the following descriptions the species of the various larvæ referred to are indicated by the letters used for that purpose in my systematic description of them (Gravely, 1909).

Spionid A ("Claparède's Unknown Larva of Spio," McIntosh, 1894).

This larva may serve as a general type, and so will be described in more detail than the rest. It was originally

described by Claparède (1863, pp. 77-80, Pl. VI, figs. 1-11), whose figures of the special structures of the anterior end are, however, very incomplete. In McIntosh's note on this larva they are mentioned very briefly, but not figured at all.

A pair of lateral lips (Pl. 14, fig. 5, *C.L.*) close in ventrally a somewhat funnel-shaped space in front of the true mouth (see below). This space, or "vestibule," as it may be termed, opens widely to the exterior in front on a level with the anterior end of the head, and ventrally by the space between the lips; the extent of these apertures can be regulated by movements of the lips.

The vestibule is lined throughout with cilia, 25μ in length,¹ which extend over the external (i. e. ventral) surface of the lips as far as the prototroch; this extends as a row of 60μ cilia down each side of the head on to the lips, where its cilia soon become indistinguishable from those of the ciliated area of the lips. At least one stout (4μ thick by 45μ long) (?) sensory cilium is present on the inner side of each lip (fig. 5, *C. st.*); its spasmodic movements, as well as its size, distinguish it at once from the other cilia.

The true mouth opens into the œsophagus from the posterior end of the vestibule; it is often exposed to view by the drawing aside of the lips. A broad band of neurotrochal 40μ cilia extends forwards across the first two segments to join the general ciliation of the vestibule beside the mouth.

On the peristomial segment minute cilia extend from beside the neurotroch obliquely forward towards a short row of 60μ cilia that is situated just below and in front of the first tuft of setæ (fig. 5).

On the second segment, the mid-ventral region of which is prolonged posteriorly, pressing back the third segment to

¹ The difficulties in measuring the lengths of active cilia are often very great. The measurements given in this paper are simply used as a means of comparing, more completely than could be done by employing exclusively the limited number of applicable adjectives of comparison, the various lengths of the cilia situated on different parts of the body; they must not be regarded as accurate measurements.

make room for this enlargement, the neurotroch is sunk in a well-defined groove or pit (see fig. 5). From this a gastrotroch, somewhat broken as shown in the figure, extends across the segment on each side.

On the remaining segments there is no neurotroch. On the third segment the gastrotroch is intermediate in form between that of the second and those of the remaining segments. On the fourth and succeeding segments it consists on each side of an outer and middle row of 40μ cilia, and an inner row of much shorter and more delicate ones. The gastrotrochs become smaller and finally disappear towards the posterior unsegmented region of the larva. The anal segment, however, bears a powerful telotroch, though this does not completely encircle the body, there being a short median dorsal gap in its continuity.

No cilia are present on the dorsal surface of the intertrochal segments. The prototroch, whose continuity is interrupted ventrally by the vestibule, shows a considerable dorsal gap also. Its cilia are 60μ long, and are borne on a pair of rounded ridges that extend dorsally from the sides of the head in a posterior direction (fig. 4, *R. Pr.*); the tentacles, when they appear, are situated immediately behind these ridges. The inner and anterior edge of each of the ridges bounds one side of a groove, which opens out anteriorly and contains a row of 20μ cilia (fig. 4, *C. Gr.*). This groove is bounded on the other side by the broad median crest of the head. Two pairs of red eye-spots are present; the posterior pair are situated upon this crest, and the anterior pair upon slight lateral prominences a little anterior to the ciliated grooves. These prominences each bear a little tuft of cilia (fig. 4, *T. C.*) 20μ long, just in front of the anterior pair of eyes.

In the most advanced specimen examined all cilia had disappeared from the ventral surface of the head (see Gravelly, 1909).

Spionid D.

This larva, probably identical with Claparède's "*Larve mit rüsselartiger Oberlippe*" (Claparède, 1863; foot-

note to p. 86; Pl. VII, figs. 1, 2), shows a remarkable modification of the above-mentioned lips, and will require a separate description. The prototroch is situated but a short distance in front of the mouth, the greater part of the lips lying in front of it instead of behind as in *Spionid A*. At the anterior end the lips meet in the middle line and are prolonged into a slender snout sheathed in a similar prolongation of the prostomium. Under the stimulus of fixing reagents these snout-like structures are usually both retracted to such an extent as to be almost unrecognisable even in sections; however, I think it is almost certain that the anterior extensions of the lips are actually fused together in the middle line; and also, until very close to their termination, at least, fused to the prostomium. But from their appearance during life I am led to believe that the "snout" is free from the prostomium at the extreme anterior end. Whether it is pierced by a canal, as seemed probable from the examination of the living organism, I have been unable to determine on sections.

This snout is supported by a pair of muscle-bands, very conspicuous during life, which pass backwards and outwards in the substance of the lips towards the bases of tentacles, and are seen in sections to be continuous with a pair of muscle-bands that extend back through the whole length of the body. Near the bases of the tentacles—which arise at rather an early stage in the development of this larva—these muscle-bands pass dorsal to a pair of contorted (?) tubes, or masses of (?) tubes, that are also most clearly seen in the living specimen.

The vestibule is ciliated throughout as in *Spionid A*, but the cilia on the external surface of the lips are confined to an area (see fig. 3) which does not extend to their posterior margin nor on to their anterior prolongation.

The neurotroch, as in *Spionid A*, is confined to the first two segments, but on the second it consists of a distinct anterior and posterior part separated by a region devoid of cilia; for this and other modifications of the ciliation of the

ventral surface of the anterior end see fig. 3. I am not able to give a detailed description of the dorsal surface of the head; as in *Spionid A*, however, the prototroch does not extend across it, but is incomplete dorsally as well as ventrally. At the sides of the head the prototroch is carried outwards as a line of 60μ cilia on to the bases of the tentacles; in this *Spionid D* differs from *Spionid A*, but resembles another common form (*Spionid B*), which I am unable to describe in detail; in general *Spionid B* resembles *Spionid A*, however. From the bases of the tentacles the prototroch is continued downwards as a line of comparatively short cilia to the outer and posterior angle of the ciliated area of the external surface of the lips. These cilia were not seen in a position to allow of their length being determined, but they appeared to be of about the same length as those (20μ long) of the ciliated area of the external surface of the lips. I was unable to determine with certainty whether this row of short cilia was quite continuous with the long cilia of the prototroch or not.

Polydora A.

As an example of the *Polydoridae* we may take this, the commoner of the two species of *Polydora* larvæ found at Port Erin during July. A specimen of *Polydora B* appeared to agree closely with *Polydora A* in the arrangement of the cilia; during life I was only able to examine the ventral surface of this specimen however. The differences between the *Polydora* larva and *Spionid A* are differences of detail only. Of those of the ventral surface one of the most striking is the presence of gastrotrochs, not on every segment, but only on segments posterior to the first and denoted by "odd" numbers, except in the neighbourhood of segment 10, which bears these cilia, whilst segments 9 and 11 are devoid of them, like the "even" segments of the rest of the body. Another difference that may be noted is the shortness of the neurotroch, which does not extend as far back as even

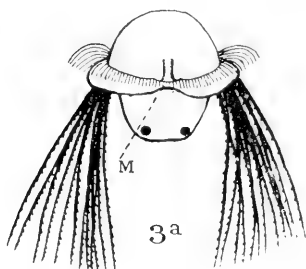
the anterior border of the second segment (see fig. 1, *Ntr.*). The stout cilia, too, of which a single pair were noted in the vestibule of *Spionid A*, were here seen to be much more numerous, though this may have been due to the position of the lips, their complete distribution from end to end of the lips (see fig. 1 of a specimen with the lips well drawn back) not being always visible even in this species. For further details of the ventral surface see Pl. 14, fig. 1. On the dorsal surface the differences between this larva and *Spionid A* are perhaps a little more striking. Nototrochs, instead of being entirely absent, are present on every segment after the second. As in *Spionid A* the tentacles arise behind the prototroch. The prototroch does not extend completely across the dorsal surface (see fig. 2), but no ciliated grooves could be found in front of it. Behind the tentacles, on the other hand, a pair of extensive ciliated grooves was found; in a 25-segment larva (the oldest examined) these grooves commenced on the posterior part of the first segment near the middle line and extended forwards and then outwards, somewhat in the form of a **U**, to the sides of the same segment slightly behind the bases of the tentacles (fig. 2, *C. Gr.*) The cilia of the prototroch are 80μ long; those of the telotroch, which is incomplete dorsally as in *Spionid A*, 100μ long.

The Development of the Vestibule and its Effect upon other Organs.

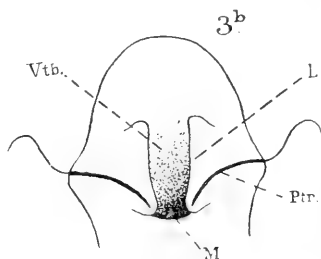
Although it would appear to be characteristic of the nectosoma stage of *Spioniform* larvæ to bear a pair of large lateral lips closing in a "vestibule" in front of the true mouth, these are not present in the earliest stages; they are absent, for instance, in the metatrochophore of *Spionid C* of the Port Erin larvæ, a species of which, unfortunately, no other stage is yet known. I have not been able to study their development at all fully, but the accompanying diagram of the anterior end of a metatrochophore of a species of *Spio*

from Port Erin (for a further description of this larva see Gravelly, 1909) will, I think, throw some light on this point, especially when compared with one of Claparède's figures of the trochophore stage of his *Polydora* (*Leucodora*) larvæ, from which the accompanying trochophore diagram is taken (Claparède, loc. cit.). From the latter it will be seen that the ridge bearing the prototroch (which is complete at this stage as in the typical "trochophore larva") is already being somewhat pressed aside in the immediate neighbourhood of the mouth. This process would appear to be continued until a gap is formed in the ridge, the proto-

TEXT-FIG. 3A.



TEXT-FIG. 3B.



- A. Trochophore of *Polydora* (after Claparède). B. Anterior end of *Spio* larva from Port Erin. L. Lip. M. Mouth. Ptr. Prototroch. Vtb. Vestibule.

troch disappearing as such in this region and being replaced by an area of short cilia. The margins of this gap form the lips, whose further growth is mainly in an anterior direction, and this brings us to the stage illustrated by the metatrochophore of *Spio*. It will be noticed that in this the now incomplete prototroch has become somewhat drawn back on to the posterior part of the lips; and this, as well as my observations upon older larvæ, leads me to believe that it forms the outer and posterior boundary of the ciliated area of the lips.

By the further enlargement of the lips it is now an easy step to the condition found in the typical *Nectosoma*, where

the original mouth of the trochophore opens, not direct to the exterior, but into a "vestibule" closed in by the lips. It is interesting to note that in *Spionid D*, in which the anterior parts of the lips are prolonged and specially modified, the part of them situated posterior to the prototroch is much smaller than in either of the more typical forms, *Spionid A* and *Polydora A*, with the result that the posterior margin of their ciliated areas extends from its lateral termination inwards and forwards, instead of inwards and backwards, as in the other two forms described, the whole of the lips appearing to be drawn forwards in every possible way.

The presence of the vestibule in front of the mouth in larvæ of the *Spionidæ* and *Polydoridae* causes of necessity a ventral gap in the prototroch; and, correlated with this perhaps, we find—apparently with equal constancy—an extensive dorsal break in its continuity. When the prototroch is thus confined to the sides of the head, its efficiency as an organ of locomotion must be seriously diminished, even when, as in some of these larvæ, its length is increased by lateral extension on to the bases of the tentacles, and this no doubt accounts, in some measure at least, for the importance of the telotroch in these larvæ, its cilia being at least as long as those of the prototroch, and often longer—a great contrast to their usual insignificance and frequent absence in the *Nereidiformia*, in which the prototroch appears to be always complete. It should be noted in this connection, however, that the most elongated of the *Nereidiform* larvæ that I have examined—that of *Nephthys*—also bears a more powerful telotroch than prototroch.

In *Spioniform* larvæ with the prototroch confined to the sides of the head too, interparatrochs are invariably present, and have frequently, if not always, become very definitely specialised, the greater part of their strength being concentrated near the sides of the body, where the cilia are very much longer than they are across the middle; they are also frequently confined to either the dorsal or the ventral surface, and even here their continuity is often broken (see figs. 3

and 5, *G.*). Thus in the Polydoridæ nototrochs are present on every segment after the second, but I have been unable to determine with certainty the presence of any cilia at all between the lateral rows of long cilia; gastrotrochs, on the other hand, are confined to segments 3, 5, 7, 10, 13, 15, 17, etc., and although complete, the cilia across the middle of the body are very small and inconspicuous. In the Spionidæ nototrochs appear to be entirely absent in many species, but gastrotrochs occur on every segment, broken into short lengths as shown in fig. 5, and always with some long cilia at each end.

Another peculiarity of the ciliation of the larvæ of the Spionidæ and Polydoridæ would appear to be the presence of a slight gap in the course of the telotroch in the mid-dorsal line. This gap is very small and often difficult to determine with certainty, but by careful watching of the cilia I have been able to find it in every species in which I have paid special attention to the point.

SOME GENERAL CONSIDERATIONS ON THE CLASSIFICATION OF POLYCHÆT LARVÆ.

Polychæt larvæ were originally arranged by Müller and others in the groups Monotrochæ, Telotrochæ, Mesotrochæ, Polytrochæ, and Atrochæ, according to the ciliated bands borne by them. Claparède (1863, pp. 84–88) has pointed out some of the difficulties which render this classification unsatisfactory, and has proposed to divide these larvæ primarily into the Metachæta, with provisional setæ, and Perrenichæta, without them, and to subdivide these classes according to the nature of the ciliated bands—the former into Gastrotrochæ, Nototrochæ, and Amphitrochæ, and the latter into Cephalotrochæ, Polytrochæ, and Atrochæ.

Agassiz (1867, p. 252) has briefly pointed out the value of the class Metachæta, and the almost certainly provisional nature of the rest of this classification, but does not make any

further suggestions. Claparède's division, *Metachætæ*, is found to coincide almost exactly with the sub-order *Spioniformia*, exclusive of the very highly modified *Chætopteridæ*, which form a distinct group to themselves, and differ from all other known *Polychæt* larvæ in being mesotrochal. But the respective characteristics, if there be any, of the larvæ belonging to the remaining *Polychæt* orders are still unknown.

Within the orders *Spioniformia* and *Nereidiformia*, to which my own observations have up to the present been almost entirely confined, certain characters can, however, be selected which appear to be diagnostic of the larvæ belonging to individual families, and the contrast between larvæ of the *Nereidiformia* and *Spioniformia* may be found in other structures besides the setæ.

Before going on to discuss these characters it will be well to consider some interesting points bearing on the phylogenetic values to be assigned to the different larval structures that appear to be most useful for systematic purposes.

Häcker (1896, pp. 93-100) has done good service in showing the effect of environment upon the form of the larva. He points out that larvæ which pass through the early part of their development in a gelatinous capsule tend to pass through a protrochophore stage; and that larvæ which pass into the later stages of development thus protected omit the true trochophore stage from their life-history, the broad band of short cilia characteristic of the protrochophore being retained by the metatrochophore; such worms may, moreover, commence their lives on the sea-bottom at an early period without ever having developed a true prototroch. Häcker (*loc. cit.*, p. 98) further finds that larvæ developed in a brood-pouch appear at the surface of the sea with a fully developed powerful prototroch. In connection with this Malaquin's work on *Syllid* larvæ may be noted. He finds (1893, pp. 424, 425) that hastened development causes the suppression of the anterior paratrochs, and in extreme cases of all the larval ciliated bands, including even the prototroch. It is also noted by Häcker (*loc. cit.*, pp. 97, 98) that the stage at which

different larvæ begin their life on the sea-bottom varies very greatly in different species; and that the ratio of the length of embryonic life in the jelly or brood-pouch to that of the free-swimming larva is equally variable. And it is obvious that this must have its effect on the production and suppression of functional larval organs.

Yet in spite of the tendency, under certain conditions, towards the suppression of larval organs, it is evident that wherever the pelagic habit is maintained for any length of time the body of the larva is encircled by powerful cilia arranged in single lines, of which not more than one is normally present on each segment; and it is to these larvæ alone that I propose to refer in the following pages.

Characteristics of apparent systematic value in larvæ may be due to either of two distinct causes, which it is necessary clearly to distinguish from one another on account of their different phylogenetic values. Either they may be due to inheritance by all the members of the group whose larvæ they characterise, from a common pelagic ancestor or from the pelagic larva, specially modified through the agency of natural selection, of some common but less remote creeping ancestor; or they may be due to some correlation between adult and larval structures. This correlation may, and undoubtedly does, often show itself in the precocious development of the structures themselves; or its presence may be much less obvious at first sight. This may be illustrated by reference to the definitely modified larvæ of the highly specialised worms forming the family Chætopteridæ.

In the adult the prostomium is reduced to a minimum, a reduction which begins to show itself in the case of *Chætopterus variopedatus* Reni, (= *pergamentaceus*, Cuv.) in quite young and unsegmented larvæ (see Wilson, 1882, Pl. XXII, figs. 82, 83) and becomes more and more marked as development proceeds; thus by the precocious appearance of an adult character a larva is produced whose form renders the possession of a powerful prototroch an impossibility; and so the prototroch is entirely suppressed and replaced by a

band of powerful cilia further back on the body. In the larva of *Telepsavus costarum*, described by Claparède and Mecznirow (1869, pp. 178-181, Pl. XIV, figs. 1-1*E*), the number of segments situated in front of the mesotroch will be found to correspond to the number of segments of the anterior region of the adult (see Claparède, 1868, Pl. XX, fig. 1); thus the precise position at which the functional secondary ciliated band is situated upon the body—this band having been rendered necessary by the suppression of the prototroch on account of the reduction of the size of the prostomium—would appear to be determined by some influence correlating larval and adult characters.

We may even venture further than this, for in the *Chætopterus* larva, whose development has been described by Béraneck (1894), two mesotrochs are present; these he found to delimit one definitive segment, and this segment proved to be the first one of the second body-region, i. e. the segment which in this form is produced laterally into a pair of wing-like processes. And probably it is the differentiation of this segment from the others of the second region that has decided the position of the posterior ciliated band, just as the differentiation of the two first regions appears to have decided the position of the anterior band. That the posterior band may have been acquired more recently than the anterior is suggested by its appearance at a later stage in the ontogeny than the anterior band (see Fewkes, 1885, p. 180, Pl. III, figs. 16-18 for ? *Phyllochætopterus*, and Gravely, 1909, for ? *Chætopterus*). It is unfortunately impossible in this connection to speak with certainty of the larva of *Chætopterus variopedatus* (pergamentaceus) described by Wilson. His oldest larvæ (twelve days) give no clue to the relation between the larval and adult body-regions, and in addition leaves quite obscure the homologies of the two ciliated bands which he describes. The cilia of both these bands at first graduate into those covering the general surface; the anterior band is extremely transitory, disappearing just as the posterior band is beginning to develop,

and apparently always consisting of more than one row of cilia. Older larvæ bear only the posterior of these bands, and this in the later stages consists of a single row of powerful cilia like those of the characteristic bands of other Mesotrochæ. Whether this or the transitory anterior band corresponds to the anterior band of Fewkes', Béraneck's, and the Port Erin larvæ it is impossible to say, but as Wilson's 12-day larva is still unsegmented, and so corresponds to the monotrochal stage of Fewkes' ? *Phyllochætopterus* larvæ and the Port Erin ? *Chætopterus* larvæ, I am inclined to think that its ciliated band is probably homologous with those of these larvæ, i. e. that the posterior of the two bands described by Wilson corresponds to the anterior band described by these other authors. In the case of no mesotrochal larva other than those of *Telepsavus costarum*, *Chætopterus variopedatus* (pergamentaceus) and Béraneck's *Chætopterus* is the genus known with certainty; but, as has just been shown, in at least two of these the line of separation between the first and second body-regions of the adult is indicated in the larva by a mesotroch, and in the third no evidence is at present forthcoming on this point.

It is clear that no larval structures which are thus the outcome of the correlation between larval and adult structures can be regarded as independent positive evidence in favour of a classification itself founded upon these adult structures, whatever their practical systematic value may be.

In the following account, therefore, special note will be taken of those characters which do not appear to show such dependence on adult structure, wherever any such can be found. The extremely limited number of Polychæt larvæ which have as yet been described in any detail should be borne in mind, for it is of necessity only on this minute fraction of those which we know must exist that the following suggestions are based.

The pelagic larvæ of the Nereidiformia are characterised by the early development of the appendages in their ultimate

form. As a rule, too, the trochophore becomes a metatrochophore by the simultaneous appearance of several ("primary") body-segments whose appendages become fully developed before any additional ("secondary") segments are added; in a few species (e.g. *Phyllodocid B* of the Port Erin larvæ) it is, however, impossible to distinguish a definite number of primary segments. This character of the simultaneous development up to an advanced stage of a number of anterior segments has become so engrained in the Nereidiform stock that it very frequently happens that the parapodia of the first two or three segments develop more slowly than do those of the immediately succeeding ones instead of appearing before them. This is most clearly shown amongst Port Erin larvæ in the (undoubted) *Syllids* (see Gravely, 1909), but it is also very well marked in the case of *Nephthys*.

In this way it comes about that until all the fundamental parts of the appendages have appeared the larva remains a rather short, stout creature capable of little of the wriggling movement that more fully developed worms exhibit. The appendages of Fewkes' *Nephthys* larva (Fewkes, 1885, pp. 180-184, Pl. IV, figs. 1-12*b*) apparently acquire their full complement of parts in the 10-segment stage; the segments of larvæ belonging to this genus are less compressed than is frequently the case in the early stages of Nereidiform worms; and this larva has a more elongated form than any other that I know of at the period of the appearance of the last-developed parts of the parapodia—which, moreover, does not take place until after the development of a number of secondary segments.¹

The only case at present known in which all the segments of a Nereidiform larva are formed in succession and of considerable length would appear to be that of *Ophryotrocha* (see Braem, 1893, pp. 219-220, Pl. XI, figs. 33-36, and

¹ Fewkes himself does not distinguish between primary and secondary segments, but in the Port Erin species of *Nephthys* at least it is certain that the former begin to appear before the gill and cirrus, which have not been seen on any larva yet examined.

Korschelt, 1893, pp. 237-242, Pl. XIII, figs. 12-15); in this form the appendages project and bear permanent setæ at quite an early stage (Korschelt, loc. cit., fig. 5), as in other larvæ of the Nereidiformia, however. When the cirri develop is not definitely stated, but it would appear to be very soon after the stage shown in Korschelt's fig. 15, when the first pair of appendages only is present, for he says (p. 240) of this stage, "von den Cirren ist an den Parapodien noch nichts zu bemerken; sie erscheinen am freien Ende abgestumpft;" and states on p. 242, "Das Stadium der fig. 15 kann man als das Uebergangstadium der Larve in den Wurm bezeichnen."

The simultaneous appearance of a small number of "primary" segments is almost certainly a secondary type of development derived from a condition such as that found in *Ophryotrocha*, and it is noteworthy that this sporadic appearance in the Nereidiformia of the primitive method of segmentation of the body occurs in a species whose adult retains the segmental ciliated bands of the larva.

The above characteristics of larvæ of the Nereidiformia may perhaps be due to the strong tendency shown by worms of this order for a few anterior segments to bear special tentacular cirri—often with the suppression of the chætigerous rami of their parapodia. This tendency for several segments to take part in the process of cephalisation may very easily be connected with the way in which in Nereidiform larvæ the developmental activities are at first entirely concentrated in quite a small number of "primary" segments, the development of succeeding ones being delayed until the appendages of the primary segments have acquired their full complement of parts. The same sort of thing occurs in the larvæ of the decapod Crustacea, in which during the Zoea stage there is a sharp distinction between the functional appendages anterior to (and sometimes including) the third maxillipede, and the appendages posterior to this, which are all rudimentary or absent (see Korschelt and Heider, 'Text-book of Embryology: Invertebrates,' Part II, pp. 249, 250). As these larval characteristics of the Nereidiformia are, therefore, probably

due largely to the correlation of larval with adult structure, and there are no other characters at present known by which these larvæ can be recognised as forming a distinct group, we have as yet no embryological evidence really independent of that obtained from the adult as to the manner in which these worms have been derived from a more primitive vermiform stock.

However, in the investigation of larvæ belonging to separate families of the Nereidiformia, structures of greater interest from a systematic point of view have already been found. The families whose pelagic larvæ are most completely known are the Polynoïdæ and the Phyllodocidæ. In both of these all known Trochophores show well-marked larval structures that appear to be exclusively characteristic of the family to which they belong. In the Polynoïdæ these are the overhanging upper lip and the associated oral ciliary apparatus, the transverse akrotoch (a simple structure, however, that is likely to be found in other families when their Trochophores are better known), and perhaps also the circlet of apical cilia (see Gravely, 1909), though Häcker figures these cilia in a tuft (1894, Pl. XIV, fig. 1; 1896, Pl. III, fig. 2) or series of tufts (1896, Pl. III, fig. 1). Trochophores of the Phyllodocidæ on the other hand are characterised chiefly by their great contractility and by the ventral "hook" of cilia.¹

The Eunicidæ appear to be characterised by the omission of the true trochophore stage. As noted above this has been shown by Häcker to be due to the early development of these larvæ taking place under the protection of a gelatinous capsule.

The strictly larval characteristics of other families of the Nereidiformia have yet to be determined.

¹ Häcker has defined the characters by which Phyllodocid trochophores may be recognised (1896, pp. 84, 85); the cilia by the eyes are not, however, present in all species (see Gravely, 1909), and must certainly be removed from the list of characters distinguishing them from those of the Polynoïdæ (Häcker, loc. cit., p. 85; see Gravely, loc. cit.).

In the Spioniformia all the segments are commonly developed in series, as in Ophryotrocha amongst the Nereidiformia, there being no distinct "primary" segments; and where any such segments can be recognised they are very few in number (e.g. three, in *Polydora ciliata*—see Leschke, 1903, Pl. VI, figs. 3–5). Moreover the appendages do not develop any adult characteristics until a large number of segments have been formed, being preceded by tufts of long and often deeply-toothed provisional setæ,¹ those situated on the first segment being longer than any of the others, and often appearing before the commencement of segmentation in species with a free-swimming trochophore stage (e.g. Claparède's *Polydora* [= *Leucodora*] larva, 1863, Pl. VII, figs. 4, 5; and Fewke's *Spio* larva, 1885, Pl. II, fig. 3). There is at present no evidence to show that these larval setæ are in any way the result of the correlation between adult and larval characters; they appear rather to be purely larval organs developed for purposes of defence or buoyancy. Their defensive function has been urged by Fewkes (1885, p. 168), because immediately upon irritation, either by a fixing fluid or an encounter with some obstacle to progression, their usual position flat against the side of the body is changed for an erect one, when the larva bristles with spines in a manner that might well defy any delicate pelagic organism. In spite of its bristles, many of which, though somewhat slender, considerably exceed the length of its body, several of the Port Erin *Spio* larvæ (see Gravelly, 1909) have, however, been seen in the intestine of the peculiarly delicate-looking larva of *Magelona*; and in view of this it may be suggested that the longer and more slender forms of provisional setæ assist in floating, as do the spines of diatoms, for any stimulus which causes the erection of the spines also causes the larva to cease from its regular movements of progression and so to sink,

¹ Claparède and Meeznikow's 'Unbestimmte Spionidenlarven' (1869, pp. 13–15, pl. xiii, figs. 1–1 *F*) does not develop these provisional setæ, but I know of no other case of their absence in a free-swimming Spionid larva.

and under these circumstances this may be the direct cause of the erection of the spines.

That the appearance of these provisional setæ in the trochophore is primitive seems hardly likely in view of the evidence, furnished by Polygordius, of the acquisition by the ancestors of the Annelida of a vermiform body before the appearance of any parapodial structures; it is more probable that they first appeared in the larva of some ancestor of the Spioniformia. At first, we may suppose, there was a tendency in these forms towards the precocious development of the normal setæ of the adult; and this was favoured by natural selection for purposes of defence or buoyancy in the water. In the presence of these setæ throughout the order Spioniformia¹—with the exception of the Chætopteridæ and Claparède and Mecznirow's 'Unbestimmte Spionidenlarven' (see footnote, p. 622)—we have then some definite embryological evidence, independent of adult characteristics, that this order originated from some definite ancestral stock distinct from that of the Nereidiformia. Claparède, it is true, includes the larva of *Odontosyllis gibba* in his list of the Metachætæ (1863, p. 87), but I think it may reasonably be doubted whether the first-formed setæ of this larva can be definitely grouped as provisional and distinctly different from their successors.

There is little evidence, however, to show that in the provisional setæ of the Spioniformia we have a better representation to-day than in their permanent setæ of the setæ

¹ The larvæ ascribed by Fewkes to the genus *Aricidea* (1885, pp. 174-176, pl. ii, figs. 4-6, and pl. vi, figs. 1 and 10), possess provisional setæ like those found in larvæ of the Spioniformia. I have been unable to consult any account of the adult characteristics of this genus, but the name suggests a close relationship with *Aricia*, a genus included by Benham ('Cambridge Natural History,' vol. ii, p. 321) in the order Nereidiformia. Fuchs ('Die Topographie des Blutgefässsystems der Chätopoden,' Jena, 1907, p. 12), on the other hand, refers the *Ariciidae* to the Spioniformia. Fewkes' larvæ of *Aricidea* are only referred to that genus provisionally. It would be of great interest to know definitely whether free-swimming larvæ of the family *Ariciidae* do or do not bear provisional setæ.

characteristic of the ancestral stock of the order, for whilst natural selection was acting on the setæ of worms that had finished their larval pelagic life and taken to the bottom, it must also have been acting in another way on those of the larvæ; and the differentiation of the two distinct types may be due to both these causes equally. Agassiz, indeed, says (1867, p. 254): "The presence of temporary bristles of huge size in the young of so many Annelids is a feature of the greatest interest from a palæontological point of view. We find repeated in Annelids the same striking coincidence between certain features only embryonic in the present types and characters of the adults in past geological time. I was particularly struck with this coincidence when examining a series of drawings of fossil Annelids kindly shown me by Mr. O. C. Marsh, of New Haven, which were all provided with bunches or single bristles of these large, rough setæ, entirely out of proportion to the width of the body, and similar to those found in the embryonic Annelids we have noticed." But there appears to have been no confirmation of this important point since its publication.

Other larval characteristics found in two families (the Spionidæ and the Polydoridæ) of the Spioniformia are the presence of decidedly specialised interparatrochs and of a vestibule from which the true mouth opens into the œsophagus as described above. In larvæ of the Chætopteridæ and Magelonidæ this vestibule is not found, but their gaping funnel-like mouths (for the early development of Chætopterus see Wilson, 1882, pp. 283-286, Pl. XXII, figs. 63-84, and Pl. XXIII, figs. 6-8, and for the later development of the same genus see Béraneck, 1894; for the early stage that alone has a funnel-like mouth in Magelona see Claparède, 1863, pp. 74-75, Pl. X, fig. 9) may easily have resulted from an exaggeration of the vestibule as found in other Spioniform larvæ.

So little is known of the larva of Magelona before the complete loss of the cilia and funnel-like form of the mouth (Claparède appears to be the only investigator who

has yet seen the larvæ before this has occurred) that it is impossible to discuss them further. They possess, however, and retain until long after the disappearance of the cilia, provisional setæ of exactly the same type as those found in larvæ of the Spionidæ and Polydoridæ.

The remarkable larvæ of the Chætopteridæ, which do not bear provisional setæ, and which differ from all other known Polychæt larvæ in being mesotrochal, have been fully discussed above.

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REFERENCE LETTERS.

C. Gr. Ciliated groove (pretentacular in *Spionid A*, post-tentacular in *Polydora B*). *C.L.* Ciliated area of external surface of lips. *C.st.* Stout (sensory?) cilia of lips. *E.* Eyespots. *Gtr.* Gastrotroch. *M.* Mouth. *Msl.* Muscle-band in lips of *Spionid D*. *Ntr.* Neurotroch. *Pr.* Anterior end of prostomium (modified to form a sheath for the “snout” in *Spionid D*). *Ptr.* Prototroch. *R.Ptr.* Ridge bearing prototroch. *S. per.* Permanent (neuropodial) setæ. *S. prov.* Provisional setæ (only the bases shown). *Sn.* “Snout” of *Spionid D*. *T.* Tentacle. *T.C.* Tuft of cilia. *Tb.* Contorted ? tubes in *Spionid D*. *Vtb.* Vestibule.

EXPLANATION OF PLATE 14,

Illustrating Mr. F. H. Gravely's "Studies on Polychæt Larvæ."

The figures have all been prepared from rough sketches of the living organism.

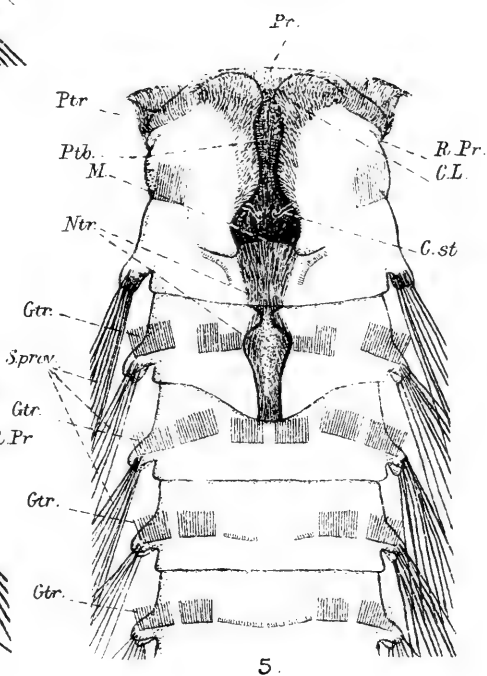
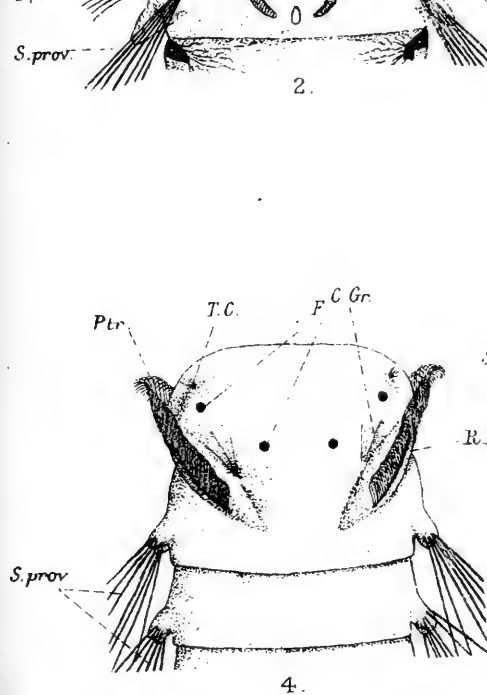
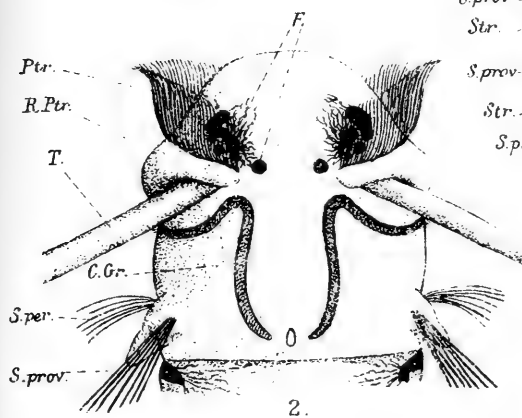
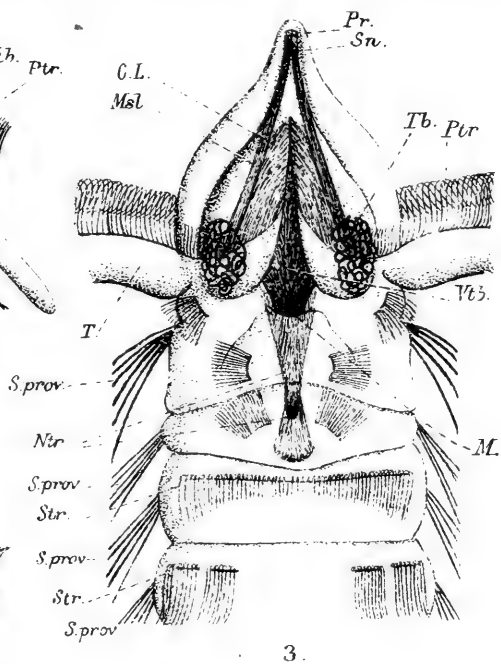
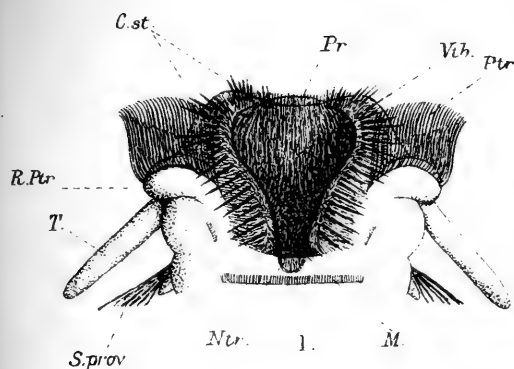
FIG. 1.—*Polydora* larva. Ventral aspect of the anterior end, with the lips drawn widely apart. $\times 130$.

FIG. 2.—*Polydora* larva, somewhat older than the last. Dorsal aspect of the anterior end. $\times 130$.

FIG. 3.—*Spionid* D. Ventral aspect of the anterior end. $\times 160$.

FIG. 4.—*Spionid* A. Stage previous to the differentiation of the parapodia of segments 7-11 from the rest; the tentacles have not yet appeared. Dorsal aspect of the anterior end. $\times 100$.

FIG. 5.—*Spionid* A. Same stage as the last. Ventral aspect of the anterior end. $\times 100$.



Some Observations on Acinetaria.

By

C. H. Martin, B.A.,

Demonstrator in Zoology at Glasgow University.

With Plate 15, and 6 Text-figures.

Part 3.—The Dimorphism of Ophryodendron.

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1. INTRODUCTION.

Instances of heteromorphism are fairly common amongst Protozoa, especially in the case of parasitic forms, but in all the known cases, as far as I am aware, where this occurs in free living forms, the differences are comparatively slight. These differences can often be connected with the Schizogonous and Amphigonous stages of a complicated life history (e. g. the megalosphæric and microsphaeric forms of Foraminifera), whereas in the case of parasites, in which the differ-

ences are often very great, there is frequently the additional factor of the change of host (e. g. *Hæmosporidia*). In the case of the animal *Ophryodendron* of which some description is given in the following pages, such an explanation of the dimorphism in terms of the Amphigonous and Schizogonous stages of a life cycle is absolutely excluded. For although conjugation has never been described in this form (Koch's theory will be dealt with later), yet from the presence of a micronucleus there is very reason to predict, that as in other *Acinetaria*, conjugation will be found to occur of a kind absolutely similar to the process of conjugation in the *Ciliates*.

And it is again interesting to observe that *Ophryodendron* is the only free living heterokaryote (i. e. *Ciliate* or *Acinetarian*)—in which dimorphism¹ has been observed.

Ophryodendron is a somewhat aberrant *Acinetarian* frequently found as an ectoparasite on *Hydroids*, and also, though more rarely, on *Crustacea*. As has long been known, it occurs under two remarkably different forms; (*a*) the proboscidiiform individual, and (*b*) the vermiform or Lageniform individual.

The proboscidiiform individual is characterised (1) by the more or less pyriform shape of the body (in *Ophryodendron sertulariæ* the body is compressed),

(2) By the absence of a stalk (this may be present in some forms, e. g. *Ophryodendron trinacria*), and

(3) By the presence of a long, very contractile proboscis furnished with rows of tentacles.

The vermiform individual is characterised—

(1) By its elongate cylindrical body,

(2) By the presence of a long solid stalk which passes into the posterior end of the body, and

(3) By the absence of a proboscis.

It is obvious that the difference between the two dimorphic forms in the case of *Ophryodendron* is very great (so

¹ By the term dimorphism the occurrence of two different reproductive forms in the same species is here meant.

great in fact that two at least among its observers, Strethill Wright and Robin, have described the vermiform animal as a parasite), in the one case as a Gregarine, in the other as a Nematode worm, but before proceeding to give any further account of the life-history of the animal, it will be necessary to examine shortly the very discrepant views as to the relations between the two individuals put forward by earlier workers on this animal.

2. HISTORICAL.

Ophryodendron abietinum was first discovered by Claparède and Lachmann in 1855 on *Campanularia* from the North Sea. Their account of this animal is in many respects by far the best which has yet appeared since they saw and figured the free ciliated embryo.

On the other hand, although they recognised the difference in external form between the vermiform and the probosciform individuals, they finally concluded that in the vermiform individuals the proboscis was retracted, and that therefore there was no fundamental difference between the two forms (p. 143, "l'extrémité antérieure de ces espèces de vers présentait une espèce d'enfoncement spécial, que nous crûmes d'abord devoir considérer comme une bouche ou comme une ventouse de succion, mais que nous reconnûmes bientôt n'être qu'une fossette indiquant l'ouverture d'une cavité dans laquelle était logé un long organe rétractile que nous aurons à décrire plus loin").

They recognised in the interior of some animals both of the vermiform and probosciform type small corpuscles "tout à fait semblables aux organes urticants des *Campanulaires*" (p. 144), but as all their efforts to surprise the animal at the moment of feeding were vain (p. 145), they concluded that "les corpuscles particuliers qu'ils renferment sont peut être comparable aux trichocystes d'autres infusoires." They thought it probable that the probosciform individuals could

bud externally, but were not in a position to positively affirm this statement.

They describe and figure two forms of ciliated embryos, a large and a small, but as in each case the buds were freed from the parent by pressure they were never able to follow their development into the fixed form.

In 1859 Strethill Wright described shortly under the name of *Corethra sertulariæ* the species at present known as *Ophryodendron sertulariæ*. In two further short papers he identified his form wrongly with the *Ophryodendron abietinum* described by Claparède and Lachmann, and added some further details on the movements of the animal.

Strethill Wright was at first inclined to regard the vermiform individuals as Gregarine Parasites, but in his later paper he considered that they were probably gemmæ. He also figures in his paper in the 'Annals and Magazine of Natural History' for 1861, the ciliate embryo, which he "freed from the parent form by a somewhat troublesome midwifery," and described very fully the movements of the proboscis.

In 1873 Hincks published some observations on *Ophryodendron abietinum*, and on a new species *Ophryodendron pedicellatum*. He was the first observer to point out clearly that *Ophryodendron* is a dimorphic form, and that (p. 4) the vermiform individual cannot be regarded as a proboscidiform individual with a retracted proboscis. He says (p. 8), "If my view of the history then be correct, the *Ophryodendron* is a dimorphic animal, that which may be called the primary zooid giving origin by gemmation to bodies unlike itself which, on becoming free, group themselves around the parent organism and lead with it an associated life." Hincks failed to see the ciliated embryo, and could find no trace of any corpuscle resembling the thread cells of the hydroid even in *Ophryodendron abietinum*.

In 1876 von Koch published a paper on a supposed new species, *Ophryodendron pedunculatum*, which he found on *Plumularia* from Messina. (This species is probably

synonomous with Hinck's *Ophryodendron pedicellatum*). von Koch describes the proboscidiiform and vermiform individuals which he terms respectively forms A and B. He does not describe the ciliated embryos, but he puts forward the novel view that the cases of association between the individuals A and B which Claparède and Lachmann and Hincks considered as probable cases of budding, were rather to be regarded in the inverse order, as the gradual stages of a complete copulation between the individual A and B, which would probably result in the formation of internal buds, as may be seen from the following passage: "Aus den Embryonen entwickeln sich die zwei verschieden gestalteten Formen A und B. B (the vermiform individual) lost sich nach eine gewisse Zeit von seinem Stiel ab, und es verschmilzt Protoplasma und Kern mit denselben Theilen von A (the proboscidiiform individual). Nach dieser Verschmelzung werden von A und B endogene Embryonen erzeugt. Gegen diese Deutung lässt sich aus meinen Beobachtungen keine Einwendung machen."

Koch's principal reason for this interpretation seems to have been the fact that he could not recognise any trace of the stalk in the full-grown vermiform individuals which he saw in contact with the proboscidiiform.

The reason for this, as will be shown later, is that the stalk is usually only formed after the vermiform individual has become free from the proboscidiiform parent.

Fraipont, in 1877, returned to the original view of Claparède and Lachmann, that the vermiform individuals are a stage in the development of the proboscidiiform individuals, though he describes the vermiform individuals as characterised "par l'absence de trompe proprement dite, et de sucoirs prehenseurs" (p. 783). It is very difficult to form a clear conception as to his views on external budding. On p. 791 he states that, "Les Proboscidiens donnent naissance par bourgeonnement externe à des individus semblables à eux soit directement soit après qu'ils ont passé par la phase d'individus Lagèniiformes." Whereas on p. 789 the following

passage is found :—"Constatons d'abord que l'on ne trouve jamais chez mon espèce des individus Lagèniiformes fixés sur les Proboscidiens."

Fraipont failed to see the ciliated buds (p. 785, "Quant à moi, je n'ai remarqué chez mon espèce la reproduction gemmipare"), and he regards the corpuscles found in the animal as "un produit du protoplasme de l'organisme et qu'ils doivent être comparés aux trichocystes que l'on connaît chez plusieurs infusoires" (p. 778).

Robin in 1879 published an account of an *Ophryodendron* under the name of *Ophryodendron abietinum*, though there can be no doubt, from his excellent figures, that the animal he observed was *Ophryodendron sertulariæ*. He considers the vermiform individual to be a true parasite, as is shown by the following passage on p. 540 :—"Les faits qui suivent montrent que cet animal est une larve d'Helminthe d'espèce encore indéterminée." He was unable to show the multicellular nature of the animal, but on p. 541 he states—"On ne saisit sur ce parasite ni bouche, ni anus, ni le tube digestif au moins en voie de formation, qu'on trouve dans les larves filariennes de beaucoup de Nématoides, auxquelles ils ressemblent morphologiquement et par la constitution de son contenu et de son tegument.

"Classer cet animal et lui donner un nomme serait risquer de faire double emploi et prémature tant que les phases de sa développement, près ou loin des *Ophryodendron* n'auront pu être suivies."

Robin was not able to see either the ciliated embryos or the nematocysts, and, although he gives accurate drawings and description of the proboscidiiform individual, he concludes that even the proboscidiiform individual cannot be regarded as an Acinetarian because the tentacles "n'ont aucun des caractères des rayons ou sucoirs des acinètes" (p. 536).

Saville Kent, in his monograph of the Infusoria, published in 1882, agrees with Fraipont that "the non-proboscidiiform or vermiform zooids must be regarded as the larval or transitional condition of the fully developed zooids provided with

their characteristic probosces. . . . Although the further development of the vermiform into the proboscidiform zooids has not so far been determined, it is clear that little beyond the everting of the neck-like anterior region of the former is requisite to bring about such a result."

As regards the process of feeding, Saville Kent put forward a somewhat novel view (p. 850):—"No evidence has, however, yet been adduced showing that these filaments or the extensile proboscis itself possesses a similar suctorial capacity, nor indeed is it known in what manner the animal grasps or incepts its food. Pending the satisfactory elucidation of this most important point, it seems most reasonable to premise that food substances are seized by the brush-like filamentous tuft or distal end of the proboscis itself, and then withdrawn with it into the parenchyma of the body."

As regards the ciliate embryos and the nematocysts, Saville Kent seems to have made no original observations; but he describes the latter as "navicula-shaped bodies" which "are apparently of an adventitious nature."

Gruber, in his account of the Protozoa of the Harbour of Genoa (1884), described a new species—*Acineta* (*Ophryodendron*) *trinacria*—attached to a copepod. He puts forward no theory as to the relations between the vermiform and proboscidiform individuals, but notes the absence of nematocysts. This form is shortly described under the name of *Acineta trinacria* by Daday, who found it on a copepod, *Tisbe furcata*, in the Bay of Naples, and he again apparently regards the vermiform individuals as developmental stages of the proboscidiform individual, although he does not bring forward any evidence to support this view.

In 1886 Milne published a short paper in the 'Proceedings of the Glasgow Philosophical Society' on *Ophryodendron trinacria*, which he described as the type of a new genus, *Stylostoma Forrestii*. The paper is of very unequal value, since he regards the macronucleus as an ovary which can be fertilised by fragments of Nucleoli; but there is one

valuable observation showing that *Ophryodendron trinacria* feeds upon free swimming ciliate infusoria.

He considers the vermiform zooids "to be immature and midway between the ciliated embryo and the adult arm-bearing form," but cannot bring forward any proof of this theory.

In 1889 Bütschli gave an excellent summary of the earlier literature in his account of the Protozoa in Bronn's 'Thierreich'; he had apparently no opportunity of examining the animal himself, but after a careful account of the conjugation theory of Koch, and the parasitic theories of Strethill Wright and Robin, finally accepts Hincks' theory of dimorphism as an explanation of the relations between the vermiform and proboscidiform individuals.

On p. 1916 he says: "Es scheint mir aber keine Bedingung der Knospungshypothese zu sein, dass die Form B (vermiform individual), in A (proboscidiform individual) übergehe, vielmehr deutet wohl alles darauf hin, dass es sich um zweierlei dimorphe Individuen handelt. Bedenklich macht mich namentlich auch die Erfahrung, dass bei den übrigen Suctorien, wie gesagt, die geschlechtlichen Verjüngungsprocesse partielle Conjugationen sind, während es sich hier entschieden um einfache Copulation handelte, wenn Koch's Deutung richtig wäre. . . .

"Gegen die Knospungslehre spricht namentlich, dass bei ihrer Annahme zweierlei wesentlich verschiedene Fortpflanzungsvorgänge bei *Ophryodendron* vorkämen, wofür keine andere Suctorie sichere Analogien bietet. Doch ist auch dieser Umstand nicht so gewichtig, da ja *Ophryodendron* auch die einzige Gattung mit dimorphen Individuen ist. Ohne Analogie wäre es ferner, dass die freien Knospen nicht in den Schwärmerzustand übergingen.

"Doch spinnen wir diese, bei der Unvollständigkeit der Beobachtungen doch resultatlosen Erwägungen nicht weiter aus. Hätte sich einer der Beobachter bemüht die angeblichen Knospen längere Zeit fortdauernd zu verfolgen, so wäre wohl

die langathmige Erörterung unnöthig geworden. Hoffentlich wird dieses bald nachgeholt."

Sand, in his 'Étude Monographique sur le Groupe des Infusoires Tentaculifères,' published in 1901, returns to Claparède and Lachmann's original view. On p. 76 he states:—"Nous croyons tout simplement qu'il n'y a pas plus de différence entre un Proboscidien et un Lagèniforme qu'entre un Dendrocometes dont les bras sont étalés et un Dendrocometes qui les a retractés. Souvent il est vrai la forme extérieure des deux variétés d'Ophryodendron n'est pas identique mais ce caractère n'est pas constant: nous avons vu des Lagèniformes identiques à des Proboscidiens et des Proboscidiens analogues à des Lagèniformes."

He did not succeed in watching the ciliate embryos or the animal feeding, but he states (p. 35): "D'après nos observations, les corpuscules naviculaires d'Ophryodendron belgicum sont identiques, comme dimensions et comme aspect aux granulations de l'ectosarc des Hydroïdes sur lesquels les Ophryodendron sont fixés."

To his further observations, especially those dealing with the structure of the vermiform individual, with most of which I can find no point of agreement, it will be necessary to return in the special part.

3. MATERIALS AND METHODS.

It will now be necessary to give my own observations on Ophryodendron, most of which were, to a large extent, controlled by work on the living animal. Most of the work was done on Ophryodendron abietinum (Claparède and Lachmann) which was found growing on Clytia during the months of July and August at Plymouth, and on Obelia during the months of October, November, and December at Millport in a particularly mild winter.

I should like to thank the staff at both these laboratories for the readiness with which they assisted me in obtaining material, and for the facilities they gave me for working it

through. I have also examined *Ophryodendron trinacria*, which I found on a copepod (*Tisbe*) at Naples, and I am indebted to Mr. Grosvenor, of New College, Oxford, for some preparations of *Ophryodendron multicapitatum* from an *Idotea* found at Plymouth, with some notes on the living animal.

The *Ophryodendron* were usually fixed with Flemming, which was washed out by H_2O_2 in 70 per cent. alcohol, or in corrosive acetic. The whole preparations were stained either with alum carmine or by borax carmine, followed in some cases, according to Hertwig's method, by picric acid. The last method was the only one by which the nematocysts could readily be demonstrated in whole preparations.

The sections were stained with hæmatoxylin followed by eosin to show the structure of the proboscis and the nematocysts.

General.

For convenience sake I have decided to divide my observations under the following headings :

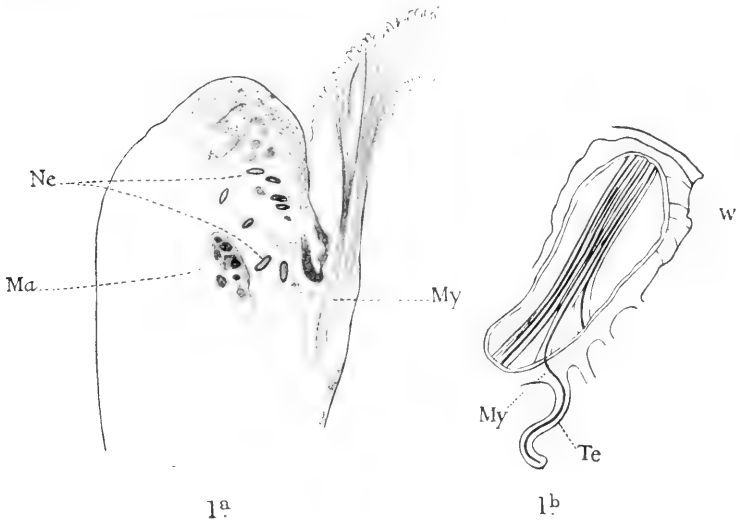
- (4) The structure of the probosciform individual.
- (5) The structure of the vermiform individual.
- (6) The feeding of the *Ophryodendron*, with notes on nematocysts in some other protozoa.
- (7) The external budding of *Ophryodendron*.
- (8) The ciliated buds.

In the following pages, unless definite reference is made to another species, the observations deal with *Ophryodendron abietinum* (Clap. and Lach).

4. THE STRUCTURE OF THE PROBOSCIDIFORM INDIVIDUAL.

The Probosciform individual of *Ophryodendron abietinum* is roughly pyriform in shape, the basal portion of the animal in the neighbourhood of its attachment being small (Pl. 15, fig. 6). In a section at right angles to the longest axis of the animal it will be seen that the animal is much

flattened in a direction at right angles to that in which the attachment of the proboscis lies, and it is probable that the proboscis arises from the surface which is ventral in the ciliate embryo. The base of the animal is directly attached to the supporting hydroid, as may be readily seen in longitudinal sections.



TEXT-FIGURE 1 *a*.—Part of a longitudinal section of a Proboscidian individual of *Ophryodendron abietinum*, to show the origin of the proboscis.

1 *b*.—Part of a longitudinal section of the distal portion of a proboscis to show the relations of the tentacles to the proboscis.

Ma. Macronucleus. *My*. Myonemata. *Ne*. Nematocysts.
Te. Tentacles. *W*. Wall of proboscis.

The proboscis takes its origin rather low down on one surface of the animal (text-fig. 1*a*), and passes forward between two lateral thickenings which I shall term the apical lobes. It is from these apical lobes that the vermiform buds take their origin. During life the proboscis is in constant motion, expanding and contracting rapidly. In an ordinary Proboscidian individual the proboscis in the contracted condition measures about 66μ , but when fully expanded

it can attain a length of over $332\ \mu$. In the contracted condition the outer wall of the proboscis is thrown into a series of wrinkled folds, and as a rule only the tentacles at the anterior end are visible; but when the proboscis is fully expanded these folds disappear, and the proboscis then is seen as a long ribbon-shaped structure with a row of tentacles on either side down the greater part of its length.

As a rule even in the expanded proboscis only the apical tentacles are fully expanded, but a series of short knobs can be seen down the rest of the proboscis with the exception of a short basal portion indicating the positions of the retracted proximal tentacles (Pl. 15, figs. 1 and 2). As the proboscis moves backwards and forwards, the apical tentacles move also, actively to and fro, so that the anterior end of a contracting or expanding proboscis looks rather like a portion of an active centipede.

In sections of the proboscis each tentacle is found to pass as a continuous tube down the whole length of the proboscis (text-fig. 1a). Near the origin of the proboscis a large number of bands, which stain very lightly in eosin, arise. A single band passes up each tentacle tube in the proboscis, and is probably instrumental in the shortening of the tentacles and the proboscis (text-fig. 1b). These bands seem analogous to the myonemes of the stalk of a Vorticellid, and similar structures can be found in sections of the tentacles of other acinetaria, e. g. *Ephelota*.

The investigation of the nuclei is rendered very difficult in fully grown forms by the presence of numerous masses of chromatin, the so-called Tinctin-körper in the cytoplasm. The origin of these masses from the nuclei of the cells ingested during the process of feeding will be dealt with in a later section. In a young proboscidiiform individual the macronucleus is a rod-shaped structure lying parallel to the animal's long axis, in the later stages of growth it generally becomes more or less T-shaped, the two branches passing up into the apical lobes.

In the young individuals a single micronucleus can always

be found lying near the distal end of the macronucleus. In section the macronucleus of both the proboscidiiform and the vermiform individuals is found to possess a distinct membrane. In sections of material fixed in both Flemming and corrosive acetic the chromatin in the resting nucleus is massed in a number of minute granules, an arrangement which seems quite different from that in any acinetarian I have hitherto examined.

In well-fed individuals the vacuolar cytoplasm is completely blocked with nematocysts, the origin of which will be described in the section on feeding.

In *Ophryodendron sertulariæ* the body is more or less disc-shaped, with a marked flattening on its lower surface (text-figure 6a). The proboscis takes its origin from the lower surface of the animal, and bends upwards to end freely in the bunch of tentacles. This is by far the most common of all the species of *Ophryodendron*, but its extremely flattened form, coupled with the fact that it usually lies closely applied to the theca of a sertularian in such a way that only the edge of the animal is presented to the observer, render it an exceedingly unsatisfactory object for detailed work.

I have only had one opportunity of examining *Ophryodendron trinacria* in a living condition.

The proboscidiiform individual is more cylindrical than the proboscidiiform individual of *Ophryodendron abietinum*, and the three proboscides arise near the distal end. The proboscis does not show any trace of the wrinkling which is so characteristic of the contracted proboscis of the other species of *Ophryodendron*, and, in fact, seems to approach far more closely a simple apical prolongation bearing tentacles such as is found in some species of *Acineta*. The tentacles are rather long, few in number, and distinctly knobbed. There is a short hollow stalk, of quite a different structure to the solid rod-like stalk which will be described in the section on the vermiform individual.

I have never seen *Ophryodendron multicapitatum* in

the living condition, but in stained preparations the numerous proboscides seemed to resemble the proboscis of *O. sertulariæ*.

5. STRUCTURE OF THE VERMIFORM INDIVIDUAL.

The fully grown vermiform individual of *Ophryodendron abietinum* is roughly cylindrical in shape, tapering somewhat towards the free distal end. The stalk is a solid rod, attached at its basal end by a slight thickening to the hydrotheca of the hydroid, and passing in the opposite direction to end in a sharp point buried in the cytoplasm of the *Ophryodendron*. During life the animal is in almost constant motion, swinging in various directions on its stalk.

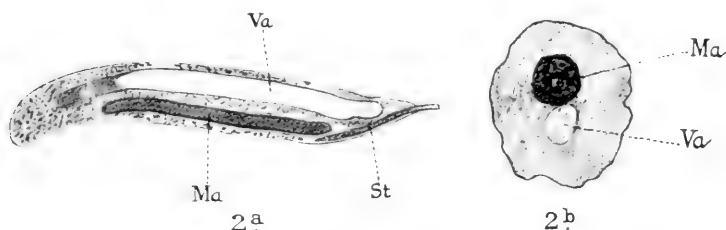
It is extremely difficult to make out the structure of the anterior end, the shape of which in the living animal is constantly changing. At one time the anterior end will show a distinct thin lip surrounding a large cavity, a little later this lip may be rolled back over the free surface of the animal, then the whole apical end may become swollen and the lip disappear.

That this lip can actually exert a powerful sucking action is shown by some observations on vermiform individuals which were still attached by their basal end to their proboscidiform parents. Under these conditions the vermiform individual would frequently attach its anterior end to the stalk of the hydroid and pull its parent over to one side. If the anterior end of the vermiform individual is carefully examined, a slender tube is found opening to the surface in the centre of the depression, and at the other end into a long vacuole, which in sections is seen to pass almost to the proximal end of the animal (text-fig. 2).

It is possibly worth noting that the first sign of disintegration in a living proboscidiform individual is furnished by the appearance of drops of cytoplasm at the end of the tentacles, whereas in the vermiform individual a similar

drop makes its appearance on the free apical surface. The cytoplasm of the vermiform individual may be crowded with the nematocysts and "Tinctin-körper" which have been previously mentioned in the account of the proboscoidiform individual. The macronucleus is usually a more or less dumbbell-shaped structure lying generally rather to one side in the posterior half of the animal.

The vermiform individuals of *Ophryodendron sertulariæ* and *Ophryodendron multicapitatum* (vide Saville Kent, p. 855) closely resemble in shape and move-



TEXT-FIGURE 2 a.—Oblique longitudinal section through the basal portion of a vermiform individual of *Ophryodendron abietinum*, showing the stalk (*St*), macronucleus (*Ma*), and long vacuole (*Va*). (2 Searcher, comp. oc.+2 mm. apochromat.)

2 b.—Transverse section through a vermiform individual, showing macronucleus (*Ma*), and vacuole (*Va*). (6 comp. oc.+2 mm. apochromat.)

ments the vermiform individuals of *Ophryodendron abietinum*, from which the vermiform individual of *O. sertulariæ* only differs in the fact that the internal end of the stalk may end in short hooks (vide Robin).

The vermiform individual of *Ophryodendron trinacria* seems, however, in the case of the few individuals which I examined, to possess a rather peculiar method of movement, by which the animal becomes contracted into a short spiral, and then slowly elongated again.

There is one curious feature in Milne's account of the vermiform individual of *O. trinacria*, the presence of a series of "setæ or cilia" at the anterior end of the vermi-

form individual. In one of Milne's figures, three of these structures are shown, and in another eight, but on the only occasion on which I examined a living *Ophryodendron trinacria* I saw no trace of these structures.

It is now necessary to refer to a rather remarkable statement by Sand that the vermiform individual of *Ophryodendron belgicum* is attached, not by a rod-like stalk but by a tentacle (p. 336). "Le pedicule du Lagéniforme est chez cette espèce un tentacule ordinaire capité, analogue à celui d'un suceur quelconque." This species was first found by Fraipont and it is rather remarkable that no mention is made in his long account of *Ophryodendron* of this remarkable structure.

Saville Kent remarks (p. 853) that "apart from its size (*Ophryodendron abietinum*, P. 1.75"—1.30". V. 1.50" to 1.30". *O. belgicum*, P. 1.400". V. 1.400") and habitat (*O. belgicum* is described as occurring on *Clytia volubilis*) the chief distinction between this type and *O. abietinum* would seem to subsist in the less luxuriant development of the tentacular appendages of the proboscis."

Bütschli also believed that the species *Ophryodendron belgicum* was identical with *Ophryodendron abietinum*. I myself worked partly on a form occurring on *Clytia* and partly on a form occurring on *Obelia*, and could find no essential difference between them and until further evidence is adduced than Sand's Pl. 13, fig. 9, it would seem impossible to credit this very abnormal state of affairs, especially as the structure figured looks far more like a stalk than a tentacle.

6. THE FEEDING OF OPHRYODENDRON.

Ophryodendron abietinum is to a very large extent a true external parasite of the hydroid to which it is attached, as will be shown by the observations on the living animal detailed below. I have, however, on two occasions, seen it

attack and suck small ciliate infusorians, the tentacles of the proboscis behaving in the same way as the ordinary acinetan tentacle.

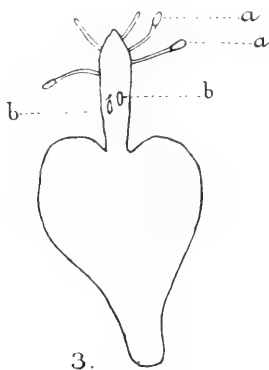
It is probable that the food of the species of *Ophryodendron* which live upon crustacea is derived entirely from ciliate protozoa in this way, as has been shown by Milne (loc. cit.). *Ophryodendron sertulariæ*, in its method of feeding would seem to resemble *Ophryodendron abietinum*, though it is probable that it is not so exclusively parasitic in its diet as *Ophryodendron abietinum*.

I found *Ophryodendron sertulariæ* growing on *Sertularia pumila* in 30 fathoms at Plymouth in July crowded with the nematocysts of *Sertularia*, and the nematocysts were very common in *Ophryodendron sertulariæ* collected from the shore at Millport in November. On the other hand, there were very few nematocysts in some *Ophryodendron* of the same species collected from the shore at Plymouth in July.

On examining fixed preparations of *Ophryodendron abietinum* by far the greater number of individuals is found grouped around the opening of the hydrotheca of the hydroid, but a few are found scattered over the main stem. In the case of the latter, it is at first sight rather difficult to believe that the tentacles of the *Ophryodendron* can reach the ectoderm of the hydroid, but it must be remembered that the tentacles of the hydroid in the living condition are usually held back well over the base of the hydrotheca, and that the proboscis which in the living resting form of *Ophryodendron* measured about 66μ in length, when expanded measured 332μ .

In an *Ophryodendron abietinum* which was drawn while feeding, it was noticed that the tentacles of the *Ophryodendron* were wrapped around the tentacles of the hydroid. After a short time the proboscis of the *Ophryodendron* was retracted, and the nematoblasts with their contained nematocysts could be seen sticking for some time in the aperture of the tentacles, giving the tentacles a curious knobbed appearance. It is this appearance that is possibly responsible for the figures of knobbed tentacles in *Ophryodendron*.

The nematoblasts could now be seen passing down the proboscis into the body of the animal with a peculiar gliding motion. In the course of this passage the long axis of the nematocyst was always parallel to the long axis of the proboscis; and when the nematoblasts passed simultaneously down the proboscis they followed parallel paths, thus indicating a feature that has already been described in the sections of the proboscis, the prolongation of the tentacles as separate



TEXT-FIGURE 3.—Living feeding Proboscoidiform individual with contracted proboscis, only a few of the tentacles are shown. *a.* Nematoblasts still fixed in the aperture of the tentacles. *b.* Nematoblasts passing down the proboscis.

tubes down the proboscis. The first stage in feeding is shown in Pl. 15, figs. 1 and 2, in which one nematoblast has been pulled out of its position in the ectoderm of the hydroid, the later stage is shown in a drawing from a living specimen, text-fig. 3, and from a stained preparation, Pl. 15, fig. 3. It would seem that the size of the nematoblasts prevents their passage down the tentacles as long as the proboscis is in its fully extended condition.

After passing down the proboscis the ingested ectodermal cells may be found (Pl. 15, fig. 3) lying in the cytoplasm of the Ophryodendron, and in some cases the whole body is absolutely blocked by them. The cytoplasm of these cells seems to undergo fairly rapid digestion, but the nucleus is far

more resistant; in early stages the nucleus retains its characteristic shield-shape and vacuolar appearance, but under the influence of the digestive enzyme its structure breaks down, and finally the only trace left of it is a number of dots of darkly-staining matter lying in small vacuoles dotted through the cytoplasm of the animal. (The characteristic Tinctin-körper of the Acinetaria.)

There seems to me a strong probability that the so-called chromidia of many protozoa may possess a similar origin from the remains of the nuclei of their prey, and I believe that a really careful study of this process of digestion of a typical metazoan nucleus in the cell-body of a protozoan might have a salutary influence on some of the extreme upholders of the chromidial hypothesis.

The nematocysts which remain after the nematoblast itself has been completely digested can readily be seen in the living animals, and can, by crushing the Ophryodendron, be readily exploded. In whole preparations stained with carmine and picric acid, and in sections stained with hæmatoxylin followed by eosin, the nematocysts are easily seen. In some cases the whole cytoplasm of the Ophryodendron is absolutely blocked with them (text-fig. 1.), and it is noticeable in such cases that the embryonic mass cut off in the formation of the ciliated embryos, which will be described later, is relatively far freer from nematocysts than the remaining husk of the parent individual.

Whether the adult Ophryodendron has any means of ridding itself of these structures must remain an open question, but on one occasion I found a proboscidiiform individual which had thrown off a mass of cytoplasm containing an enormous number of nematocysts which had exploded on contact with water. This individual seemed perfectly healthy next morning, so that it is possible that the process is a normal one. This is the only occasion on which I have found a free Ophryodendron with exploded nematocysts.

It is thus clear that the nematocysts of Ophryodendron are derived from its host, and this explains the fact that

nematocysts have never been found in the other species of *Ophryodendron* which live upon Crustacea.

The case of the nematocysts of *Ophryodendron* is, of course, paralleled by that of the nematocysts of *Æolids*, as has been shown by Grosvenor, and by that of the nematocysts of *Turbellaria*. I have had an opportunity of observing two analogous cases amongst *Infusoria* :—

(1.) In some *Kerona* which were found on a rather morbid *Hydra* at Glasgow, the three characteristic *Hydra* nematocysts were present.

(2.) During a stay at Naples I found that a holotrichous infusorian on *Eudendrium*, which seemed to agree in all essentials with the *Holostoma* described by Entz (*loc. cit.*), was full of the two very characteristic nematocysts of the hydroid in an unexploded condition.

It is far more difficult to arrive at a clear conception of the process of feeding by direct observation in the case of the vermiform individual. During life the vermiform individual is in constant swaying motion on its stalk, touching with its anterior extremity all objects within reach. That the anterior end of the vermiform individual possesses considerable power of suction is shown by the fact that an external vermiform bud which has not yet become completely detached from the parent probosciform individual, can often be seen applying its anterior end to the stalk of the hydroid, and pulling its parent right over. At the same time, nematocysts are always found in the vermiform individual, and in fixed preparations I have found cases in which a vermiform individual had its anterior end closely applied to the ectoderm of a hydroid, whilst the nuclei of freshly ingested ectodermal cells were to be seen in its cytoplasm.

7. THE EXTERNAL BUDDING OF OPHRYODENDRON.

In following the external budding of *Ophryodendron* on the living animal, as far as my experience goes it is essential that the *Ophryodendron* should be kept in a fairly deep

watch-glass, and not under a coverslip. In a typical case a short piece of hydroid was placed in a flat-bottomed watch-glass at 5 p.m. on Saturday, and the positions and appearance of all the *Ophryodendron* on it were carefully noted. The proboscidiiform individual showed the commencement of the formation of a vermiform bud. On Sunday morning the vermiform bud was fully grown, but was still in direct continuity with the proboscidiiform. At 5 p.m. on Sunday the vermiform bud was active, and was almost free, and at 5.30 p.m. it was quite free and attached to the hydroid stem.

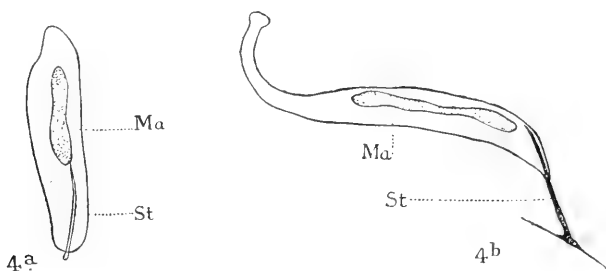
The young, free vermiform individual can travel considerable distances in a leech-like fashion, using its ends as suckers. The observations were repeated on other individuals with the same result. The internal details of this process of budding are shown in Pl. 15, figs. 4 and 5.

In Pl. 15, fig. 4, a proboscidiiform individual is shown, in which the right apical lobe is prolonged, indicating the first stage in the formation of an external vermiform bud; on the left side there is a fully developed vermiform bud, which has not yet become free.

In section it is easy to see that the bud is formed as a hollow outgrowth, a fact which explains the rapidity of the early stages in its development, as well as the enormous apparent disparity in mass which is sometimes seen between the bud and its proboscidiiform parent.

In Pl. 15, fig. 5, the last stage in the division of the macronucleus between the proboscidiiform and the vermiform bud is shown. At this stage the vermiform individual is always much swollen at its apical end, and it is only later that the vermiform individual becomes elongated, and a distinct lip is developed round the anterior end. The young vermiform bud now becomes active, and finally pulls itself away from the parent individual. At this stage the vermiform individual creeps up the stem of the hydroid, finally becoming attached by its posterior end and developing its characteristic rod-like stalk. At first the stalk is quite short, and the greater length is hidden in the posterior end of the animal

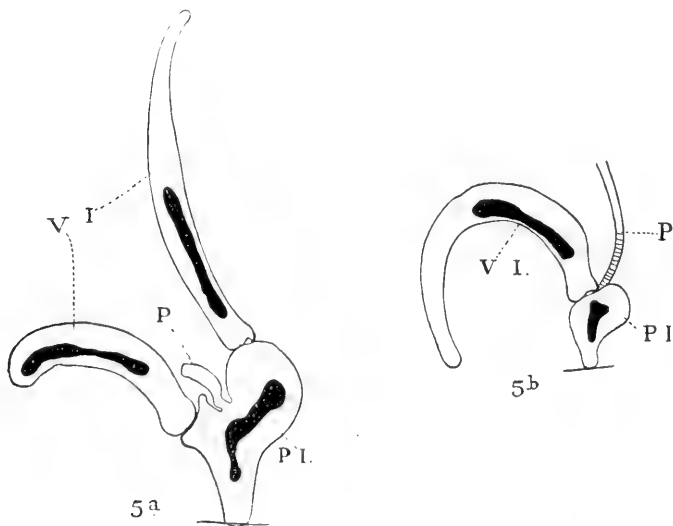
(text-fig. 4a), but later it elongates and assumes the appearance characteristic of the adult individual. I do not know whether the adult vermiform individual can leave its stalk



TEXT-FIGURE 4.—Vermiform individuals of *Ophryodendron abietinum*.

4 a.—Showing development of the rod-like stalk (*St.*).

4 b.—With fully developed stalk. *Ma.* Macronucleus. (2 Searcher, comp. oc. + 2 mm., apochromat.).



TEXT-FIGURE 5 a.—A large Probosciform individual with a late Vermiform bud on the right side, and an earlier Vermiform bud on the left.

5 b.—A small Probosciform individual with a late Vermiform bud on the left side.

P. Proboscis. *P.I.* Probosciform individual. *V.I.* Vermiform bud. (± comp. oc. + 4 mm. apochromat.).

and wander to a fresh position, but the bare stalks of the vermiform individual are often found attached to the hydroid.

Fraipont and Sand's view that the vermiform individual can develop into a proboscidiiform individual will be examined in the concluding section, but it may be well to state here that I have never been able, either in continuous observations on the living animal or in fixed preparations to find the slightest evidence for this transformation.

There is one interesting fact as regards the budding of the vermiform individual which I think points clearly to the conclusion that the vermiform individual is a distinct dimorphic individual. The size of the proboscidiiform individual is very variable, whereas that of the budded vermiform individual is singularly constant even in those cases in which the parent proboscidiiform individual is quite small (see text-fig. 5).

This absence of correlation between the size of the parent proboscidiiform and that of the attached vermiform bud is shown in the following table¹:

	Proboscidiiform individual.		Vermiform bud.	
	Greatest length of the body, excluding the proboscis.	Greatest breadth.	Greatest length.	Greatest breadth.
Small Proboscidiiform .	34	20	138	11
Medium Proboscidiiform .	48	33	142	23
Large Proboscidiiform .	92	46	184	13

These figures do not really express the difference in mass between the two individuals since the vermiform individual is cylindrical, and its width is more or less uniform throughout the greater part of the animal's length, whereas in the proboscidiiform individual, owing to its pyriform shape, the greatest width is far greater than the mean width.

¹ Measurements in μ .

The only method of reproduction I have found in the vermiform individual is by the formation of internal ciliated buds, which will be described later (Pl. 15, fig. 10).

Hincks puts forward the possibility of the vermiform individual giving rise to other vermiform individuals, but the single preparation on which this interpretation is based is not convincing, and I have never been able to see anything of the kind in any of the vermiform individuals I have examined.

8. THE CILIATE EMBRYOS.

It is rather remarkable that the only observations on the free ciliated embryo are due to the earliest workers on this form—Claparède and Lachmann and Strethill Wright. In both these cases, however, the embryos were released by an operation, and it is to this fact that Claparède and Lachmann's statement that the embryos in *Ophryodendron* are of two sizes is probably due, since the large ciliated embryos divide in the broad pouch to give rise to the normal small free embryos (Pl. 15, fig. 7). Ciliate embryos are formed both in the proboscidiiform and the vermiform individuals of *Ophryodendron abietinum*. I have only seen the ciliated embryos of the proboscidiiform individual actually escape on five occasions, although, especially at Plymouth, I wasted much time in watching for it. This was partly due to the fact that a considerable period, over twelve hours, during which the embryos divide, intervenes between the time at which the cilia of the embryos in the brood pouch become active and the final escape of the embryos, and partly possibly to the evil effect of the coverslip. Probably the best method would be to keep the *Ophryodendron* in a watch-glass, but here the extremely small size of the embryos would present a great difficulty, though it is possible that this might be partly obviated by the use of a water immersion objective.

The first sign of the formation of the ciliate embryo is the rounding off of a central block of protoplasm (Pl. 15, fig. 6),

the embryonic mass which contains the greater part of the nucleus of the parent. This embryonic mass gives rise to usually about six to eight oval blocks measuring about $46\ \mu$ long, by about $14\ \mu$ wide, which develop cilia and swim actively about in the brood pouch.

A rather small proboscidiform individual was found to contain six large active ciliate embryos at 5.45 p.m., and at 8.35 p.m. one of these measuring $46\ \mu$ long was seen to show signs of a division, which was complete by 10 p.m., the two products of the division measuring $24\ \mu$ long. By the next morning all the embryos in the brood pouch had divided.

Just before the ciliate embryos escape, they exhibit paroxysms of activity, during which they swim over and over each other in the brood pouch, these periods of intense activity being interspersed by long rests, during which the cilia beat very languidly.

In the case of a small proboscidiform individual, of which the six embryos were very active at 3.30 p.m., they broke through at 5 p.m., measuring $28\ \mu$ long by $14\ \mu$ by $10\ \mu$. On the other hand, a large proboscidiform individual, seen at 4 p.m. to contain a large number—over 30—of active embryos which escaped at 4.50 p.m., and which measured $20\ \mu$ by $10\ \mu$ in the stained preparation. These embryos were fixed at once, and one of them is figured (Pl. 15, fig. 11).

The embryos seemed always to escape from a lateral opening not far from the animal's point of fixation. Nearly all the cytoplasm and nuclei is used up in the formation of the embryonic masses, and in most cases in which an individual contained a large number of embryos, the parent, after the escape of the embryos, is left a mere shell which soon perishes. The free embryos are more or less oval animals with a decidedly flattened ciliated ventral surface, and a convex dorsal surface. On the animal's left side there seems to be a flap overhanging the ventral surface. In the ventral region there are large vacuoles which often contain nematocysts, and there is a single small contractile vacuole. They move in a curious hesitating manner with the narrow end

forward, far more slowly than the ciliate embryos of *Acineta papillifera*, halting at intervals on their posterior end to make a half left turn. In the case of some embryos which were seen to escape at 5 p.m., they were almost stationary at 6.35 p.m., and were fixed at 7 p.m.

The embryos attach themselves by their posterior end, and the proboscis makes its appearance as a projection on the anterior ventral surface.

Claparède and Lachmann's drawings (11, *a* and *b*), seem to show very faithfully the appearance of the free living ciliate embryo; (*a*) being a ventral view, and (*b*) a lateral one.

I have never succeeded in observing the escape of the ciliate embryo from the vermiform individual, though I have often seen the cilia slowly beating in the large earlier embryo, and on one occasion I saw six small embryos in very active motion in a vermiform individual. The shape and size of the ciliated embryo in the latter case coincided with those of the normal free embryos developed from a proboscidiiform individual. There appears to be, however, a constant difference between the number of ciliated embryos in the vermiform and the proboscidiiform individuals; in the former I have never seen more than six embryos, whereas in the large individuals of the latter the number may be great—over 30 (Pl. 15, fig. 10).

I believe that the embryos of both the proboscidiiform and the vermiform individuals always gives rise on fixation to young proboscidiiform individuals, since while small proboscidiiform individuals of about the same bulk as the ciliate embryo are often met with (Pl. 15, fig. 12), the smallest vermiform individual I have seen with a well-developed stalk measured 115μ long by 18μ at its widest point.

RESULTS OF EARLIER WORKERS.

The chief conclusions of the earlier workers can be summarised in tabular form :—

	Relations between Probosciform and Vermiform Individuals.	Ciliate Embryo.	Nematocysts.
Claparède et Lachmann, 1855 (<i>O. abietinum</i>)	P. is identical with V. (the proboscis being retracted)	Saw two kinds	Trichocysts.
Strethill Wright, 1861 (<i>O. sertulariæ</i>)	(a) V. is a Protozoan parasite. (b) P. buds V.	Saw one kind	No mention.
Hincks, 1873 (<i>O. pedicellatum</i>)	P. buds V.; also V. buds V.	Not seen	Not seen.
Koch, 1876 (<i>O. pedunculatum</i> = <i>pedicellatum</i> Hincks)	Contact between V. and P., a process of copulation resulting in formation of ciliate	Not seen, but presumed to give rise to P. and V.	No mention.
Fraipont, 1877 (<i>O. abietinum</i>)	V. is a developing P.	Not seen	Trichocysts.
Robin, 1879	P. not a true acine- tarian. V. a parasitic worm	„	Not seen.
Kent, 1882	Agrees with Fraipont	„	Possibly ex- ternal origin.
Sand, 1901	Agrees with Fraipont	„	„

9. CONCLUSIONS.

Ophryodendron abietinum is to a large extent a true external parasite on its supporting hydroid, and the nematocysts which it contains are derived from its host on which it preys. In some cases I have seen the probosciform individual attack and suck small ciliates, and it is probable that the species of *Ophryodendron* which are attached to Crustacea obtain their nourishment entirely in this way.

The study of the nuclei is much obscured by the presence

in the animal of masses of chromatin (Tinctin-körper) derived from the nuclei of the ingested nematoblasts, but in the ciliated embryos and in the young fixed proboscidiiform individuals there is always to be found a minute sphere of chromatin surrounded by a clear area which, from its position and behaviour, must be regarded as a micronucleus.

It will now be necessary to examine the theories of the earlier workers as to the relation between the two individuals of *Ophryodendron* in the light of the observations detailed above.

- (1) The conjugation hypothesis of von Koch.
- (2) The parasitic theory of Strethill Wright and Robin.
- (3) The developmental theory of Claparède and Lachmann, Fraipont, and Sand.

(4) The dimorphic theory of Hincks and Bütschli.

(1) The Conjugation Theory of von Koch.—Von Koch, as has already been shown in the historical introduction, put forward the view that the various stages of the connection between the vermiform and the proboscidiiform individual are not to be regarded as stages of budding, but rather in the inverse order as stages in a conjugation which results in the complete fusion of the copulating individuals. This theory is excluded by the observations above made upon living forms in which it has been found that the vermiform individual is actually budded off from the proboscidiiform, the whole process from the first appearance of the bud to the setting free of the vermiform individual having been observed to occupy about twenty-four hours.

(2) The Parasitic Theory of Strethill Wright and Robin.—It is interesting to note that both the upholders of this view were working on the species of *Ophryodendron sertulariæ*, in which the vermiform and proboscidiiform individuals are so different in appearance that it is almost impossible to imagine transitional stages between them; and the very fact that this theory has been put forward must, I think, be regarded as an indication that Claparède and Lachmann's view of the identity of the two individuals is, to say the least of it, strained.

Robin's belief that the vermiform individual is a young nematode, is of course quite untenable, and Strethill Wright's view that the vermiform individual is a parasite protozoan is contradicted by:—

- (a) The fact that the nucleus of the vermiform is during the process of budding in continuity with the nucleus of the probosciform individual.
- (b) The identity of the ciliate embryos in the two forms.
- (3) The Developmental Theory.—This theory must be examined from two points of view:—

- (a) From that of the original belief of Claparède and Lachmann, that the vermiform individual possessed a retracted proboscis and was identical with the probosciform individual.
- (b) From that of its later development in the hands of Fraipont and Sand according to which the vermiform individual must be regarded as a developmental stage of the probosciform individual, this development consisting in a change in shape, the resorption of the characteristic rod-like stalk, and the formation of the proboscis.

(A) Claparède and Lachmann's view is, I think, explained by the fact that in morbid vermiform individuals which have been kept some time under a coverslip, the cytoplasm towards the anterior end can contract away from the pellicule to fill the central cavity, and thus give the appearance of a dark central mass which might be taken for a retracted proboscis. Their fig. 2, Pl. 5, which is said to represent an "*Ophryodendron abietinum* avec la trompe retractée" is quite clearly a young vermiform individual which has not yet formed its stalk.

(B) There are no observations on the living *Ophryodendron* showing the development of the vermiform individual into the probosciform individual, and though I have frequently observed the same vermiform individual at intervals for periods of over forty-eight hours, I have never been able to see any indication of such a transformation.

The figures on which Fraipont bases this view are quite unconvincing:—Fig. 31, Pl. 1, which is said to represent a “Lagèniforme ayant perdu son pédicule et dont la forme du corps se rapproche de celle d’un Proboscidiën,” is quite clearly again a young vermiform individual which has not yet developed its stalk, while fig. 10, which is said to represent a “Lagèniforme dont l’extrémité antérieure se différencie en trompe pour passer à la forme Proboscidiën,” is probably a proboscidiiform individual in a morbid condition in which, as is frequently found, the proboscis is the first portion of the body to break down.

In the case of Sand the evidence is of the same kind, but, as has been shown in the special part, even less satisfactory.

Sand states on p. 200 of his monograph:—“Lorsque la trompe et rétractée l’individu s’appelle lagèniforme, vermiforme ou individu B, il diffère quelquefois des proboscidiens ou individus A. par la forme extérieure. Cependant toutes les transitions ont été observées pour plusieurs espèces.”

The only evidence of this transition which Sand brings forward rests on a single specimen of *Ophryodendron abietinum*, which (p. 206) “a été fixe dans nos préparations au moment où ses 14 tentacules commençaient à proéminer sur un petit cercle du corps.” The specimen is again referred to on p. 74. “Or, sur un exemplaire d’*Ophryodendron belgicum* nous avons pu observer un phénomène, qu’aucun auteur n’a vu jusqu’ici: le premier stade de l’expansion de la trompe (Pl. 14, fig. 2, probably Pl. 16, fig. 2). L’animalcule en question affectait cette forme ovale et trapue qui est décrite comme caractéristique des *Ophryodendron belgicum* pourvues d’une trompe. Sur une petite zone circulaire de l’extrémité distale du corps, proéminaient 14 tentacules courts cylindriques, capités en tout semblables à ceux des autres Suceurs.

“Ce petit cercle forme, à n’en pas douter, l’extrémité distale de la trompe lorsque la partie environnante du corps s’étire pour former cet organe.”

The figure is rather a puzzling one, as the tentacles are

represented end on, and it is only safe to say that it certainly does not furnish the required evidence of the transformation of the vermiform into the proboscidiiform individual.

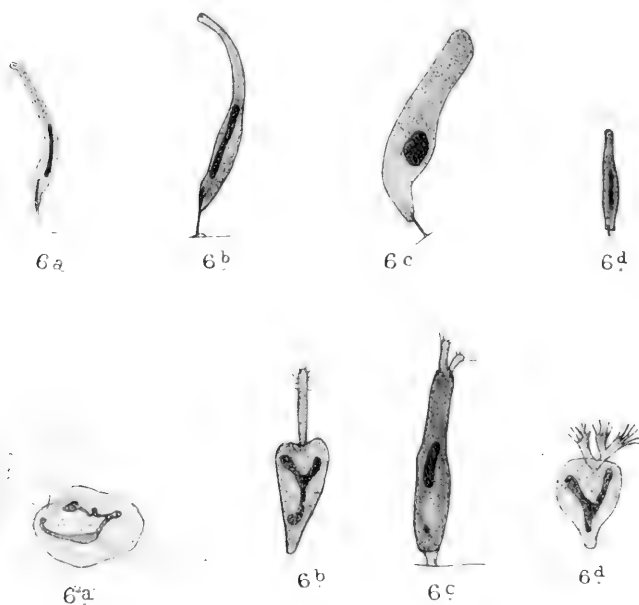
It will thus be seen that there are two main sources of error in attempting the proof of this theory from a mere examination of fixed specimens—(1) The presence of young vermiform individuals which have broken loose from the proboscidiiform individual, but have not yet developed their stalk; (2) the presence of degenerating proboscidian forms seen in a horizontal plane.

Finally the presence of active ciliated embryos in the vermiform individuals show that they can hardly be regarded as immature forms, and it will be evident, from what has been said above as to the structure of the two individuals, that the change from the vermiform individual to the proboscidiiform individual would need to be of a far more radical nature than Fraipont and Sand have imagined.

(4) Hincks' Theory of Dimorphism.—Of the various theories put forward to explain the appearance of the two individuals in *Ophryodendron*, I think that the observations given above show clearly that Hincks' theory of dimorphism is alone in accordance with the facts. The proboscidiiform individual gives rise by a process of external budding to the vermiform individual; both the proboscidiiform and the vermiform individual can give rise to ciliated buds, which are already ciliated before the process of division is complete. This last fact gives the explanation of the two sizes of ciliated embryo found by Claparède and Lachmann. I have never been able to follow the history of the ciliated buds from the vermiform individual, but from the fact that the smallest free vermiform individual that I have been able to find, after looking over many hundreds, was far larger than the largest free ciliate embryo found, I believe the ciliate embryos of both forms always develop into proboscidiiform individuals.

From an evolutionary standpoint, it would seem that the dimorphism of *Ophryodendron* presents a case which is

full of difficulties. It has always been assumed that the proboscidiiform individual represents more closely the ancestral acinetarian form, and that the vermiform individual is a more recent development. If this point of view is accepted, it is rather remarkable that in the various species of *Ophry-*



TEXT-FIGURE 6.—Diagrams of the Proboscidiiform and Vermiform individuals of *Ophryodendron sertulariæ* (A.), *O. abietinum* (B.), *O. trinaeria* (C.), and *O. multicapitatum* (D.), to show the similarity of the Vermiform individuals and the diversity of the Proboscidiiform individuals. (2 Searcher, comp. oc. + 4 mm., apochromat.).

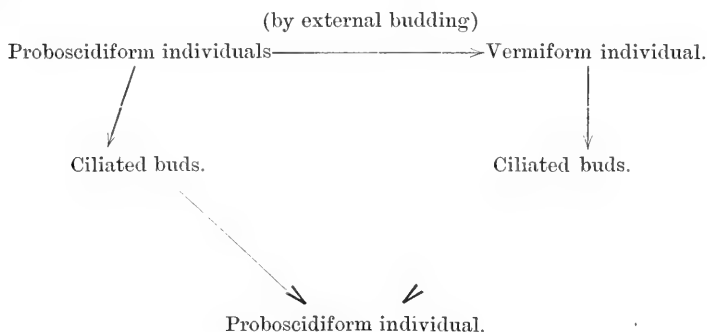
odendron the vermiform individuals are almost indistinguishable in shape, whereas the proboscidiiform individuals are absolutely different from one another (text-fig. 6). In this case, apparently, the more primitive proboscidiiform individual has undergone far more extensive changes than the more recently developed vermiform individual. It might be suggested that the vermiform individual, once it had been

produced, was in such perfect accordance with the environment, that no further change would be necessary.

It is very difficult to regard this explanation as being in any way adequate when it is remembered how different the environment of a vermiform individual of *Ophryodendron trinacria*, which is attached to a free swimming copepod, must be from that of the vermiform individual of *Ophryodendron abietinum*, which is to a large extent a true external parasite of a hydroid. On the other hand, if the number of embryos of the same type produced by these two forms is to be regarded as some measure of their success in the struggle for existence, there is distinct evidence that the vermiform type cannot be regarded as so successful a form as the proboscidiiform type.

Diagram of the life cycle.

The stages actually followed in living specimens are indicated by black arrows. The probable development of the ciliate buds of the vermiform individual is shown by a dotted line.



10. SUMMARY OF RESULTS.

- (1) *Ophryodendron abietinum* is a true ectoparasite of the hydroid to which it is attached, and its contained nematocysts are derived from its host.

This conclusion holds good for *Ophryodendron sertulariæ*.

- (2) *Ophryodendron* is a true dimorphic form, the probosciform individual (A) giving rise by a process of external budding to a vermiform individual (B) of quite different structure.
- (3) Both the probosciform and the vermiform individual can give rise to ciliate embryos.
- (4) The ciliate embryos of the probosciform develop on fixation into young probosciform individuals. It is probable that the ciliate embryos of the vermiform individuals also develop into probosciform individuals.

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EXPLANATION OF PLATE 15,

Illustrating Mr. C. H. Martin’s “Observations on Acinetaria.”
Part 3.—“The Dimorphism of *Ophryodendron*.”

ABBREVIATIONS.

C. e. Ciliate embryo. *E. M.* Embryonic mass. *Ma.* Macronucleus. *Mi.* Micronucleus. *Ne.* Nematoblast. *Ob. t.* Obelia tentacle. *Pr.* Proboscis. *Pr. I.* Probosciform individual. *Te.* Tentacle. *Ti.* Tintin-körper. *V. B.* Vermiform bud. *V. I.* Vermiform individual.

PLATE 15.

Ophryodendron abietinum.

FIG. 1.—Diagram showing probosciform with extended proboscis, some of the tentacles being attached to the tentacle of an Obelia. (4 comp. oc. + 4 mm. apochr. Zeiss.)

FIG. 2.—Details of the proboscis shown above; one nematocyst (*Ne.*) has just been dragged out of its position in the ectoderm. (6 comp. oc. + 2 mm. apochr.)

FIG. 3.—Later stage of the feeding of a probosciform individual; the proboscis is now retracted. *Ne.* = nematoblasts at the end of the tentacles. *Ne*¹ = a nematoblast on its way down the proboscis into the cytoplasm. *Ne*², *Ne*³ = stages in the digestion of the nucleus of the nematoblast. (6 comp. oc. + 2 mm. apochr.)

FIG. 4.—Probosciform which has just budded a vermiform individual on one side, and shows the beginning of a second vermiform bud on the other. (2 [Searcher] comp. oc. + 2 mm. apochr.)

FIG. 5.—The last stage in the division of the macronucleus between a proboscoidiform and a vermiform bud. (4 comp. oc. + 2 mm. apochr.)

FIG. 6.—Early stage in the development of the ciliated embryos in a proboscoidiform individual. The rounding-off of the embryonic mass. The proboscis is not retracted. (2 [Searcher] comp. oc. + 2 mm. apochr.)

FIG. 7.—Two proboscoidiform individuals showing the later stages in the development of the ciliated embryos. The proboscides are retracted. (2 [Searcher] comp. oc. + 2 mm. apochr.)

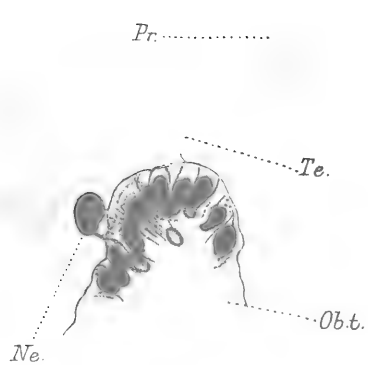
FIG. 8.—Dividing ciliate embryo from the brood-pouch of a proboscoidiform individual. (6 comp. oc. + 2 mm. apochr.)

FIG. 9.—Part of the brood-pouch of a proboscoidiform individual showing the two stages of the ciliate embryo. (2 [Searcher] comp. oc., + 2 mm. apochr.)

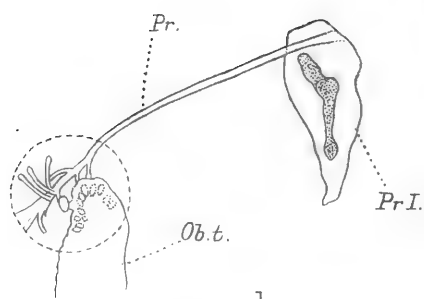
FIG. 10.—A vermiform individual containing ciliate embryos. (2 [Searcher] comp. oc. + 2 mm. apochr.)

FIG. 11.—A free ciliate embryo. (6 comp. oc. + 2 mm. apochr.)

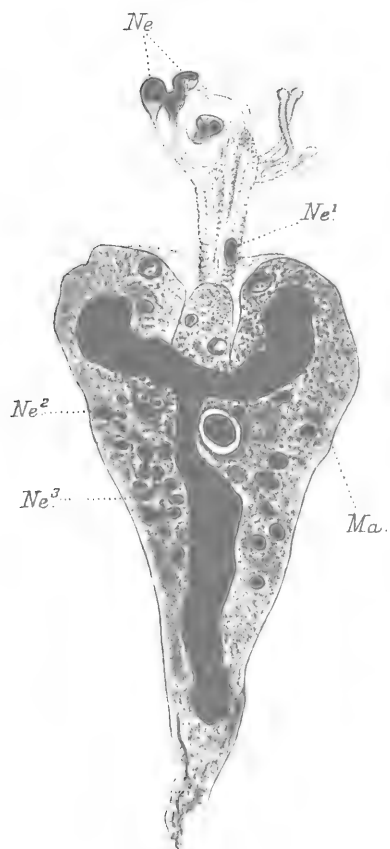
FIG. 12.—A young proboscoidiform individual. The whole of the proboscis is not drawn. (6 comp. oc. + 2 mm. apochr.)



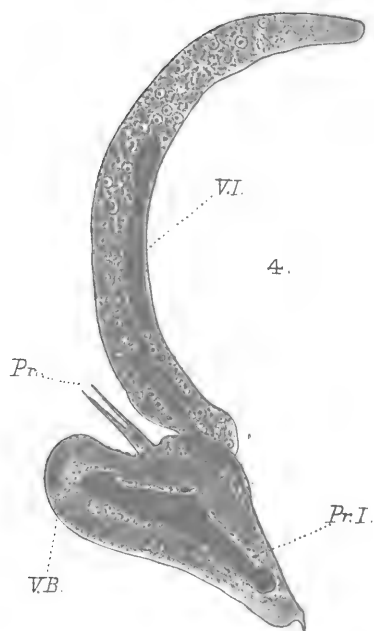
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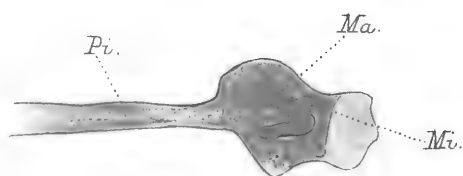
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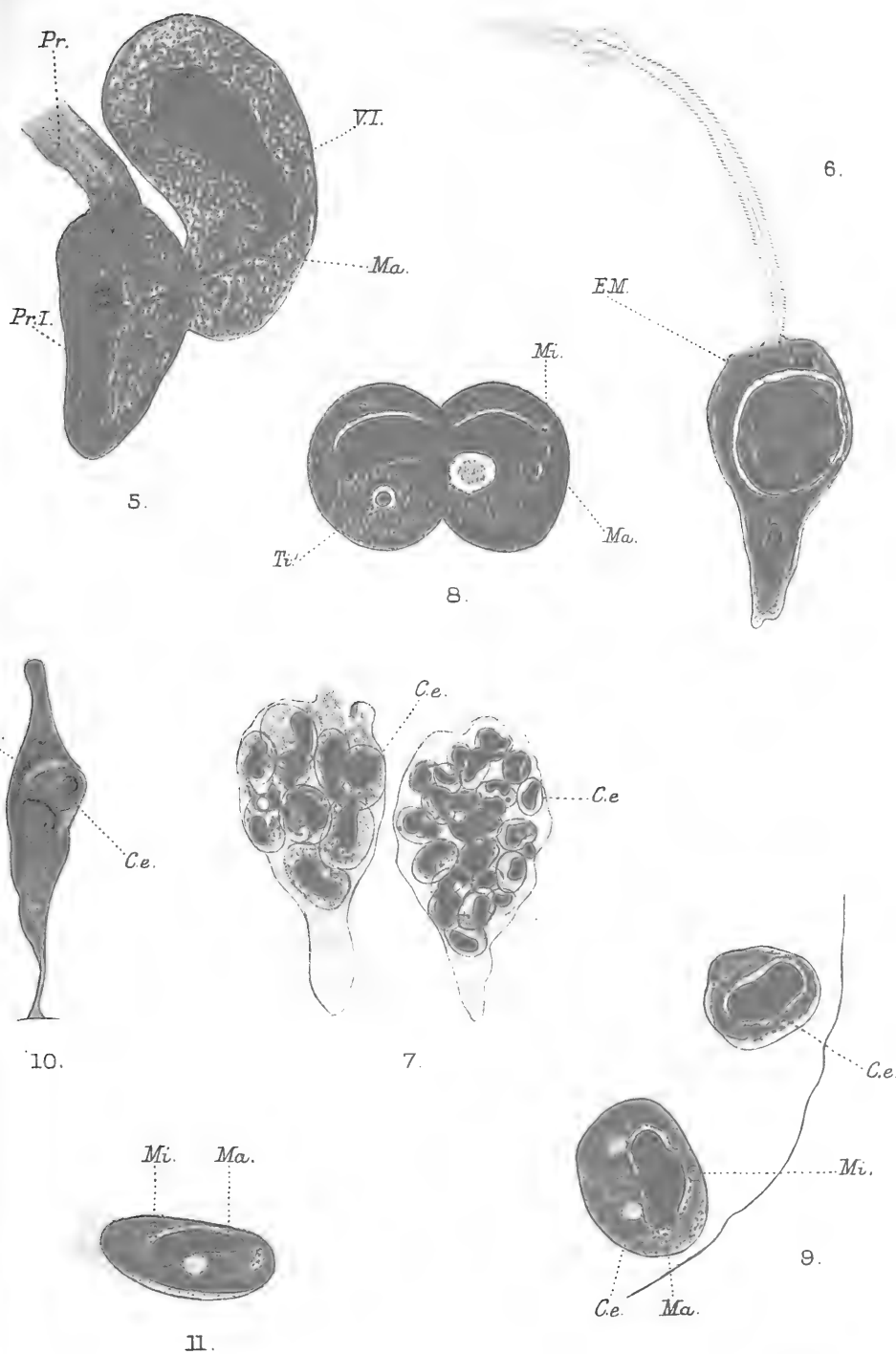
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Studies on Ceylon Hæmatozoa.

No. 1.—The Life Cycle of *Trypanosoma vittatæ*.

By

Muriel Robertson, M.A.,

Carnegie Fellow in the University of Glasgow.

With Plates 16 and 17 and 4 Text-figures.

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I. INTRODUCTION.

IN August, 1907, I went to Ceylon with a view to investigating the Protozoan blood parasites of Reptiles. The following memoir gives an account of the Trypanosome infection found in the soft Tortoise *Emyda vittata*.

I wish here to express my great indebtedness to Dr. Arthur Willey, F.R.S., Director of the Government Museum in Colombo. He not only obtained laboratory facilities for me at the museum but also gave me the most enthusiastic assistance in every possible direction. In some instances, at very considerable inconvenience to himself, he actually sent me the infected material which he had come across while engaged upon other work in the jungle. Indeed it is to him, as will appear later, that I owe the discovery of the true intermediate host in the infection which forms the subject of this paper.

Emyda vittata is a soft tortoise covered all over with soft skin, coloured black above and pure white underneath, hence its native name of "kirri ibba" or milk turtle.

The animal has flaps on the under side so arranged that all the four limbs and the head can be completely withdrawn. Like most of this group it is more or less nocturnal in its habit, it is said to leave the water very rarely, but I have myself, while watching a pool near Allutoya, where the country is all jungle, seen one come out of the water immediately after sunset and start prowling about at the edge. These soft tortoises die if they are kept out of water for more than a few hours, so that in order to carry them safely from one place to another it is necessary to wrap them in a damp cloth or put them in a wet bag of sacking or palm leaf. The creature is to be found practically all through the low country. I examined specimens from Colombo, from Kesbawa and Hanwella (both not far from Colombo) from Hambentot, which is on the eastern side, and from Trincomalee, also on the eastern side. It is, relatively speaking, common, but not nearly so abundant as the lake tortoise, *Nicoria trijuga*, as far as I could make out they were on the whole more plentiful on the eastern side than in the west.

I never heard of *Emyda vittata* being seen up country, although *Nicoria trijuga* is to be found in large numbers at Peradeniya and in the Kandyan district generally, which is at an elevation of nearly 1500 feet.

Emyda vittata is very generally infected both with a large Trypanosome (Plate 16, figs. 1—4), and with a *Hæmogregarine*. I never came across a really satisfactory negative case, although cases were observed where one or other of the parasites appeared to be absent. Protozoologists are quite familiar with the difficulty of being certain that a negative diagnosis is correct. I several times tested apparently negative specimens only to find that at last a stray parasite or two did finally turn up.

I attempted to obtain uninfected specimens by hatching out the eggs already provided with hard shells which were

found in the oviduct of a freshly-captured *Emyda*. There were five eggs but none of them hatched out, and Mr. Fernando, the taxidermist of the museum, who had, he told me, frequently tried similar experiments, said that he had never got eggs obtained in this way to hatch out. It might possibly be that the length of time that the egg spent in the oviduct before being laid had some effect upon its capability for further development. One of the eggs was opened after more than three weeks, and although it was quite well preserved no development had taken place.

As a rule the *Emyda* was fairly well infected and in some cases the blood contained a very large number of parasites. I may say at the outset that in spite of the most diligent search and of a slight theoretical bias in favour of the hypothesis I could not find any connection between the Trypanosome and the *Hæmogregarine* infections. I propose to discuss the *Hæmogregarine* in a subsequent paper.

As far as my observation goes this Trypanosome is only found in *Emyda vittata* and I have called it *Trypanosoma vittatæ*. A Trypanosome is found in the fish *Saccobranthus* (see "Some Ceylon Hæmatozoa," Drs. Castellani and Willey, 'Quart. Journ. Micr. Sci.,' vol. 49, 1905), which inhabits similar localities, but this is obviously a quite distinct species. *Nicoria trijuga*, though often to be found living side by side with *Emyda vittata*, and also as in the Colombo lake in water which harboured a very large number of the generally infected *Saccobranthus*, never showed any sign of a trypanosome infection at all. My own observations upon blood parasites in Ceylon and those of many observers upon the European forms, especially those occurring in birds and amphibians, point towards the necessity of exercising considerable care before deciding that any hæmatozoon is specific to any given vertebrate host. Nevertheless, in the present instance I feel considerable confidence in attributing the Trypanosome in question exclusively to *Emyda vittata*.

Trypanosoma vittatæ is a large form resembling

T. raia in its external appearance and partly also in its movements in rather a remarkable manner, the most striking difference being in the situation of the trophonucleus which in *T. vittata* lies much nearer to the kinetonucleus.¹

II. OBSERVATIONS UPON THE LIVE TRYPANOSOME.

A great deal of time was spent in making observations upon the living object, as it is obvious that where possible it is by far the most satisfactory method.

Trypanosoma vittata in the live state is a pyriform organism with a well developed frilled membrane. The frilled appearance is of course due to the membrane being longer from tip to tip at the free edge than at its origin from the protoplasmic body. The trophonucleus can be clearly distinguished as a circular body lying at no great distance in front of the kinetonucleus. It appears as a greyish sphere surrounded by a brighter halo: there is something very characteristic in the rather soft way in which the nuclear structures refract the light, contrasting sharply with the very hard, bright appearance of the protoplasmic inclusions—this is alike true of *Trypanosomes* and *Hæmogregarines*. Striations

¹ I have adopted the now very generally accepted terms of kinetonucleus and trophonucleus for the small and large nuclear bodies respectively. These terms seem to me to express more adequately than any of those hitherto proposed the nature and function of these two structures. In this paper the expression "anterior" end is used as equivalent to the flagellate end, "posterior" end as equivalent to non-flagellate end. The evidence in favour of this view being correct seems quite convincing when one has regard to those *Trypanosomes* in which the *Trypanosome* phase is derived from a *Crithidial* or *Herpetomonad* form in the normal life cycle. The evidence for regarding the flagellate end as the anterior is not so clearly indicated in *Trypanosomes* which adopt the *crithidial* or *herpetomonad* condition by the mere alteration in shape of the body and the migration towards the flagellate end of the already existing flagellum. This development, as is well known, is said to occur in the cultured forms of a very large number of different *Trypanosomes*, notably those of birds and mammals.

or myonemeta can be seen running longitudinally along the body; these are sometimes extraordinarily clear, and are especially conspicuous just before the animal rounds itself off.

The flagellum runs forward from the kinetonucleus—which can occasionally be distinguished in the live specimen—along the edge of the undulating membrane and ends in a long free whip. The waves of contraction which pass along it make little sharp corners appear at what may be called the bays of the frills, giving a very characteristic appearance, though one difficult to describe in words.

Bright inclusions may be present all along the body, arranged without any appearance of regularity; they are often entirely absent, and I never discovered upon what either their absence or their presence depended. The body of the Trypanosome is apparently oval in section. The posterior end extends some way beyond the kinetonucleus; it is changeable in shape, and may be drawn out to a rapidly tapering point, or be rounded off and rather blunt.

The movements of this Trypanosome are rather complicated, and it can execute a considerable number of different figures. Like many Trypanosomes its motion of translation in the blood of the vertebrate host is relatively speaking slight; this is in marked contrast to the extraordinarily rapid darting movements of certain of the forms developed in the transmitting host. Quite possibly this lesser power of actual translation through space is correlated with the fact that the parasite in the vertebrate blood is in a medium which is itself in motion. *Trypanosoma vittatæ* shows the wheel motion so often seen in Trypanosomes in fishes; it also executes repeated serpentine twisting, sometimes in a figure 8, or even following a simple U-curve down one limb and up the other. The most characteristic movement, however, is a rather slow, forward spiral twisting. The Trypanosome will sometimes go on revolving slowly round its long axis with the body in the shape of a corkscrew, and with hardly any forward motion at all. The spiral twisting often occurs rhythmically forwards and backwards, through a distance of about

twice or thrice the length of the Trypanosome. This last movement is familiar to observers who have worked with Spirochaetes, only in these it is intensely rapid, while in the case of the Trypanosomes it is quite a slow movement.

In most infections small Trypanosomes (Pl. 16, figs. 5—7) are to be seen, less than half the size of the average specimens. In these the membrane is generally a little wider relatively than in the case of the larger forms, and the part of the body posterior to the kinetonucleus is much shorter and generally more pointed. The protoplasm is usually rather hyaline. I do not think that these small specimens belong to a separate species, as forms intermediate in size are also to be seen. Further, these small creatures take part equally in the developmental process to be described presently. I am, however, ignorant of their origin.

Another variation, only rarely met with, is that some specimens have the trophonucleus very much further forward than is usual. These creatures were present in small numbers only in one infection. The ordinary forms were also present. I cannot be certain as to whether they belong to the same species or not, but am personally inclined to think that they do.

There is always a considerable variation in the size and thickness of the Trypanosomes, and also, to a certain extent, in their staining reactions; but it is not marked enough for there to be any reason, in my opinion, for dividing them into male, female, and indifferent upon their morphological characters. This type of difference between the forms is much less evident than, for instance, in such a Trypanosome as *T. brucei*.

Longitudinal division does occur, but it is only very rarely to be seen, even in good infections. Specimens with two trophonuclei are very occasionally to be seen; it so happens that I have seen these chiefly among the intermediate sized forms.

If blood infected with *Trypanosoma vittatae* is placed upon a slide, covered with a coverslip and sealed with vase-

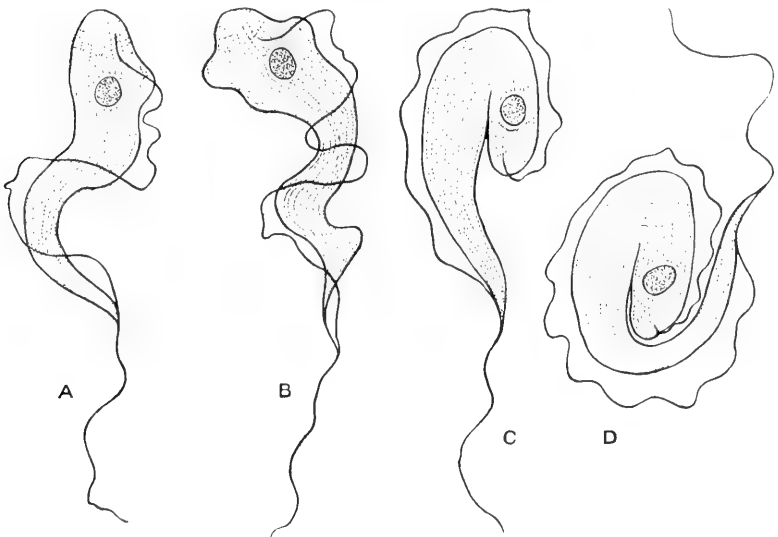
line, quite a different form of multiplication can be observed. The first time I observed this, a slide with blood very strongly infected with Hæmogregarines, and only relatively slightly with Trypanosomes, had been left overnight. Various alterations in the appearance of the Trypanosomes, to be described presently, had been noted before leaving the slide in the evening. Early next morning the slide was found to contain quite a number of small flagellates, just about the size of the Hæmogregarines and very much smaller than the Trypanosomes. A few unaltered Trypanosomes were present, but no intermediate forms. The appearance of the flagellates strongly suggested a connection with the Hæmogregarines. They had comparatively short flagella, the membrane reaching to about between one third and two thirds of the way up the protoplasmic body in different specimens. In fact, they showed a tantalising resemblance to the figure of the Trypanosome phase in the blood of the little owl, as described in Schaudinn's well known memoir, and attributed by him to the life-history of *Proteosoma noctuæ*.

The experiment was repeated several times, and the following development was made out, showing clearly that the organism arose from the Trypanosome.

Some time after making the preparation the Trypanosomes begin to show various modifications in the external appearance. The length of time which elapses before the creature begins to yield to the altered conditions is remarkably variable, the time co-efficient throughout the whole process is in fact very inconstant. Generally speaking the organisms remain unaltered for about an hour and a half. The alterations in appearance culminate by the complete loss of the Trypanosome shape and the rounding off of the organism, but this condition is arrived at in various ways. Some of the Trypanosomes simply become much thickened at the non-flagellate end. Many become bent upon themselves, and the two limbs of the bend then fuse together (text-fig. 1, c and d). The text-figures illustrate these appearances. The myonemata become much more evident in most cases during

these early phases. In some cases the animal broadens considerably and adopts the spiral shape, the turns of the spiral fuse together, and the most grotesque dumpy creature is produced, which keeps up a slow corkscrew or revolving motion (text-fig. 2).

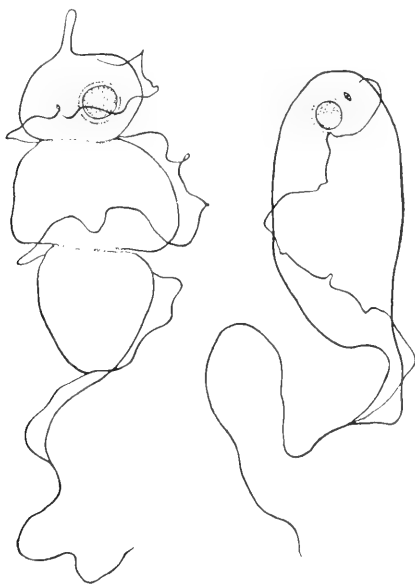
Another appearance of rather a curious character is that where the screw movement backwards and forwards is kept up but very slowly, and the body no longer preserves its



TEXT-FIG. 1.—Sketches of live Trypanosomes to show early phases in the rounding off of the parasites. A and B are one individual, so also C and D.

regular fusiform shape, but bulges now in one direction now in another (text-fig. 1, A and B). The movement is difficult to convey, but is best described as very metabolic euglenoid movement associated with a slow screwing backwards and forwards. During this movement the myonemeta can still be very clearly seen, and besides these at the non-flagellate end circumferential lines running round the creature can be seen, especially during the screw forward.

These appearances seem on the surface to show a curious amount of variation, but it is easily explained if it is remembered that at this stage an obvious decrease in the firmness of the peripheral protoplasm is taking place; in fact it becomes much more soft and viscid. This in correlation with the various methods of movement found in the normal trypanosome produces all the figures noted above. Thus, for

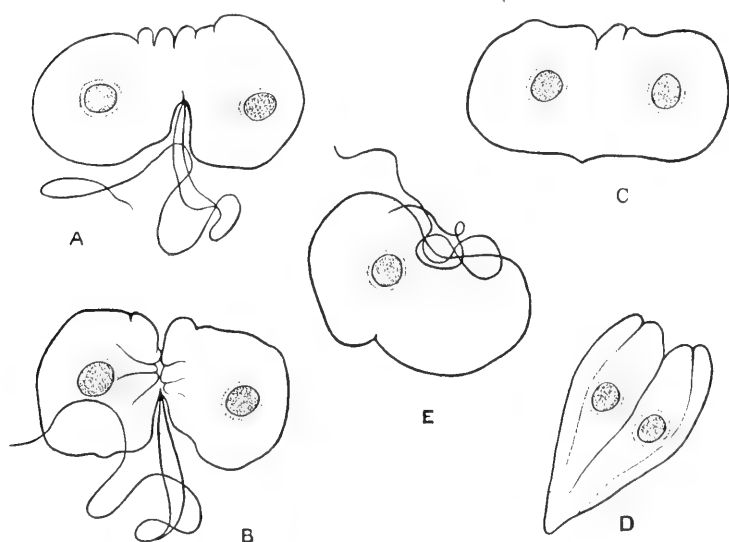


TEXT-FIG. 2.—Two different methods of rounding off.

instance, in text-fig. 1, c and d the Trypanosome has obviously been executing the wheel motion when its protoplasm began to soften, and fusion occurred, and so on.

Finally, whatever the method adopted the Trypanosome comes to rest, and the flagellum breaks loose from the membrane while retaining its attachment at the kinetoplast. It still lashes about for a time. All trace of the myonemeta completely disappear, and the animal appears as an irregular mass of protoplasm (text-fig. 3, E). Occasionally

part of the body at the extreme anterior end (flagellate end) projects—this is, as far as I could make out, not withdrawn into the body, but seems like the membrane and flagellum to disintegrate. After a time the nucleus becomes quite indistinct, and I noticed that after this I was never able to get a very clear view of the nucleus until just before the flagellate condition was again adopted, when it showed with customary distinctness. Furrows now begin to appear in the animal, and it divides into two (text-fig. 3, A and B). Another division



TEXT-FIG. 3.—Stages in the crop of the leech. A and B. Division stage of one individual. C and D. Individual adopting the pear shape at time of division. E. Newly-rounded off Trypanosome with flagellum still attached.

follows this, and four irregular rounded or pear-shaped creatures are thus formed, lying generally more or less connected. They now put out each a flagellum on one end. The flagellum is at first simply a short thick process. It lengthens and begins to lash slowly from side to side, but is as yet not capable of moving the body. Presently a slight oscillation of the body of the parasite is to be observed, and ultimately as the flagellum lengthens the creature becomes motile.

On one occasion I observed the whole process under a high power in an already pear-shaped individual. The flagellum can only be said suddenly to have appeared as a short, relatively thick process at the blunt end of the organism. This lengthened and became motile, and after a time its origin from the body appeared to lie more laterally, and a slight ridge became visible at that point. I am inclined to think that the ridge is the first appearance of the undulating membrane.

At this stage again, both as observed on the slide and when the process takes place, as will be seen later, in the leech, much variation in small detail is to be remarked, especially in relation to the relative times at which the different processes occur. Thus in the present case the preparation for the second division may, and very often does, take place before the completion of the first. Or, on the other hand, the two products of the first division may become quite separate before any preparation for the second division can be detected. In the matter of the flagella there is also much variation. Sometimes all the four flagella are developed before the first division occurs, or this may not take place until the completion of the second division. Generally speaking, the development of the flagellum lags behind when the process occurs on the sealed slide, while in the leech the flagella are developed as a rule very early.

The typical pear shape, which ultimately becomes fusiform, may be adopted very early; in fact, sometimes at the second division the protoplasmic body will adopt the form of a longitudinally-furrowed cone rounded at the broad end. These furrows are rather curious, as there may be a number of them giving the animal a ridged appearance. The deepest furrow is where the ultimate line of division occurs. The other furrows disappear. The length of the flagellum is again in some cases considerable before the body of the organism begins to lengthen at all, and rounded little creatures, with quite long flagella, may not uncommonly be seen in blood from the crop of the leech; they are of quite

rare appearance on the sealed slide of blood direct from the *Emyda vittata*.

At this stage of the investigation the question of the transmitting host came to be considered. This was the more difficult to determine as no parasites of any kind had been found on the milk tortoises. The aquatic habit of the tortoise pointed to some water inhabiting blood-sucking form, and of these the leeches seemed the most likely. A number of the common Ceylon water leech, called by the natives *Diya kudella*,¹ were investigated, but none of them showed any sign of the trypanosome. It was found, however, that the leech fed readily upon the tortoise. Upon puncturing the crop immediately after feeding it was seen that the Trypanosomes began to undergo the above-described transformation at once upon being taken into the crop.

Thus a leech was put on to a milk tortoise at 8.30 a.m.; at 10.30 it was still feeding; at 11.30 it was found free in the tank. The crop was punctured at once, and the blood examined in the usual way on a slide ringed with vaseline. The Trypanosomes were for the most part already casting off their flagella, and many of them had already undergone the first division. By 1 o'clock they were divided into four, and many of the resulting *Herpetomonas* forms had become free, and were swimming about on the slide. Blood was then taken from another part of the crop, and the state of the parasites was exactly similar to that on the sealed slide.

The puncturing of the leech does not apparently cause it much inconvenience. The specimen mentioned lived quite well till the next day, when I finally opened it at 7.30 a.m. Many actively motile forms in the Crithidial stage were to be seen.

Further divisions it seems may occur after the two mentioned, and also a secondary increase in size. Some of the individuals had become considerably lengthened, and were

¹ Mr. W. A. Harding, who kindly examined the leeches brought from Ceylon, considers this leech to be *Limnatis* (*Poecilobdella*) *granulosa*.

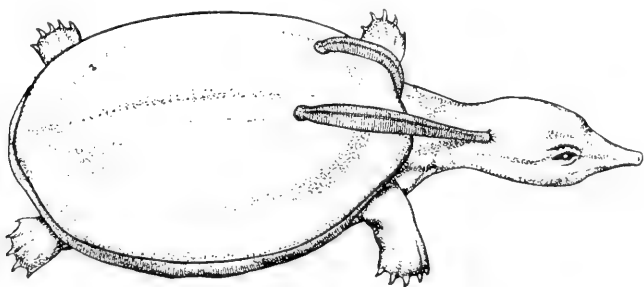
rather slender, but in almost every case the trophonucleus was posterior to the kinetonucleus.

Another leech killed ninety-five hours after feeding on the same tortoise showed slender Crithidial forms in the crop and some irregularly-shaped individuals. The intestine never showed many of the flagellates. A number of leeches were allowed to feed on various tortoises with the intention of observing the progress of the infection at different distances of time. Unfortunately, the work was at this point interrupted by illness for a number of weeks. On resuming it I found that the Trypanosomes still did persist in the crop in a few cases, but they were always very scarce. In one positive case they were to be found six weeks after feeding. An experiment was tried by feeding a leech for a second time on infected blood to see if the second infection would persist in greater numbers than the first, but by some fatality the tortoise ate the specimen that I had in this condition, and I never made out if the parasite showed any signs of becoming acclimatised to the host. The tortoise showed the greatest desire to eat the leeches, and had to be carefully watched; even so, it was surprising how often the more lively *Emydas* got the leech. They gulp them down, but do not break the skin with their teeth. The leech seems rather difficult to swallow, but the tortoise is most persevering, and it is almost impossible to rescue the leech once the tortoise has got well started.

I mention this, as there is always the possibility of parasites being spread by way of the digestive tract. The leech generally prefers to sit on the carapace attached by the posterior sucker and to fix its anterior sucker to the occiput, or back of the neck, or into the humeral angle. The accompanying text-figure is from a pencil sketch made in a few minutes by Dr. Willey, and gives a very typical picture. The leech will stretch to an almost incredible extent when the tortoise puts out its head rather than let go.

There is one point in the habits of this leech worthy of mention, namely, the fact that it will often feed in two instalments. Thus a leech would attach itself and feed for

about an hour, and then cease and move about on the carapace, or even leave the tortoise altogether, and upon being replaced would take a second meal. I was in some doubt as to whether the leech had always fed the first time, but in a few cases it certainly had, and it had generally made the characteristic little wound. This habit, I think, may have a certain importance, owing to the very rapid changes which take place in the Trypanosome in the crop. I do not wish to imply that the horse-leech is likely to be a facultative transmitting host for this particular Trypanosome in nature, but the fact is of interest in regard to the transmission of parasites by leeches generally.



TEXT-FIG. 4.—*Poecilobdella granulorum* feeding on *Emyda vittata*. (Drawn by A. K. Maxwell from a sketch by Dr. A. Willey.)

The time taken to digest a meal appears to be much shorter in the Ceylon freshwater-leech than, for instance, in such a creature as *Pontobdella muricata*, the common marine leech which infests the skate of European seas, where very many months elapse before digestion is complete. Thus one of the Ceylon leeches which had fed vigorously so that it had been seen to swell out to a great extent, was nearly empty as regards its crop after fifty-three days. The size of the leech has, of course, a good deal to do with the length of time taken to digest a meal, a big leech taking longer than a small one. I should say that about two to six months was the time taken to complete the digestion of a meal. I have no idea how long elapses in nature between the completion

of digestion and the searching for more food. All the leeches captured that I opened were empty of blood, but they could not by any means always be made to feed—I opened some of the ones that had refused, and found that they were empty. On the other hand a leech which had obviously fed on December 2nd fed again on February 1st: this beast was eaten by the tortoise, but it seemed to have been feeding and certainly left a wound. Curiously enough all the land leeches I examined were empty, and that was also Dr. Willey's experience in the jungle. This is possibly due to the fact that the fed leeches are not in search of prey, and therefore not so easily found.

On one occasion a curious example was given of how indifferent the water-leech is as to whether it feeds upon warm- or cold-blooded creatures. I had tried to make one of them feed upon a *Saccobranchus* infected with *Trypanosomes* with a view to discovering if any such change occurred as that seen in *T. vittatæ*, the leech steadily refused to feed. I then took it out of the tank, and it instantly attacked my hand making the customary little triradiate scar and drawing blood. It was made to desist, and then immediately put on to a tortoise where it quite contentedly made a large meal.

The experiments with the *Diya kudella* were now abandoned as what appears to be the true intermediate host was come upon.

Dr. Willey, while making some observations at Kesbewa, about eight miles from Colombo, found three *Emyda* with a number of small leeches attached to the back of the neck, the angles between body and limbs, and the region near the tail. He very kindly brought them to me at once. The leech is a very small creature. It attaches itself to the tortoise, but is most capricious about staying on it; I found it a most troublesome little animal to work with on that account. It is not very easy to keep it alive in captivity.

The leech belongs apparently to the genus *Glossiphonia*, and has a trick of lying together in little clumps; it is able to swim, and also covers the ground rapidly by walking

under the water on its suckers in precisely the same way that the common land leech does on land.

This leech broods its young, but it usually seems to carry about only very few with it. I have frequently got them with one or two quite good-sized young ones, and remember on one occasion taking three parents, each carrying one young one, from a tortoise, and putting them into a watch-glass. They had all got detached and mixed up in the process, a short time later they were once more arranged in pairs, but I had no means of discovering if each parent had selected out its own offspring or had just adopted the first one it met.

The *Glossiphonia* is a very inconspicuous creature, is quite aquatic, and dies very soon if left out of water, which probably accounts for it not having been found sooner. The tortoises were generally brought in by natives, and it was always some hours before they were examined, and I expect in many cases the leeches had died and dropped off before they reached the museum.

From this time forward leeches of this species were got from time to time, but it was difficult owing to the relatively free habit of the beast and small size to get them in numbers. A good number were got for me by Dr. Willey from Hambentot in the south-eastern part of the Island. They came from milk tortoises (*Emyda vittata*) living in a bathing place; alongside of them were lake tortoises (*Nicoria trijuga*) with *Ozobranchus*¹ upon them.

The leeches seem to be specific to the two tortoises, only one *Glossiphonia*, an empty specimen, was found on the top of the carapace of a *Nicoria*; its presence was probably quite casual, just as I have occasionally found a stray Branchellion on a *Tropidonotus* (water-snake) which was living in a tank with *Nicoria*—they never fed on the snake.

The digestion in the *Glossiphonia* completes itself in about two to six days, according to the size of the leech, but I do not know exactly what period of time must elapse before the

¹ *Ozobranchus*, a species of this genus of leech is found in great numbers upon *Nicoria trijuga*. Mr. Harding states that this is a new species.

animal feeds again in nature. An apparently empty leech will sometimes quite refuse not only to feed, but to remain on the tortoise. Nevertheless, from observations on captive leeches, it does not appear to me to be more than a few days. The *Glossiphonia* show a marked tendency to get into the less-exposed corners of the body, such as the folds of skin at the back of the neck, round the limb bases, and under the tail. They were actually seen to enter the cloacal chamber which is a relatively large cavity in these tortoises.

The *Glossiphonia*, in contrast to the horse-leech, shows the Trypanosome very frequently in nature, in fact, the majority of the specimens are infected. And the parasite persists in empty leeches where no coloured matter is to be detected in the alimentary tract. I was never able to find the very earliest stages of the parasite in this leech owing to the difficulty of manipulation. It was neither easy to get the leech in a condition willing to feed nor to catch it at exactly the right moment after feeding. This was, of course, due to its small size and wandering habits correlated with the exceeding rapidity of the early changes in the Trypanosome.

In the most recently-fed animals at my disposal the parasite was already in the shape of a rather broad flagellate approaching the crithidial condition—the first two divisions had, in most cases, already occurred.

Thus a *Glossiphonia* which had fed on infected blood at some time between 8 a.m. on April 6th and 7 a.m. on April 7th was opened just after the latter hour. The Trypanosomes were already mostly in the shape of crithidia, but a very few were still in the rounded state just completing division. Some long slender forms, very narrow, with pointed posterior end, and the flagellum only reaching back to a little more than the middle of the body were already present. This indicates that the development is even more rapid than in the horse-leech.

These long forms were not, I think, left over from the previous meal as they were of a type not usually found at the end of digestion.

The course of the infection in the *Glossiphonia* appears to

be in brief as follows:—The Trypanosome ingested with the blood develops in a few hours into a flagellate, rather rounded and broad in shape. It may grow very considerably in size, and adopt the Trypanosome condition, i. e. with the kinetonucleus posterior to the trophonucleus. Division still proceeds. Great variation in shape and size occurs in this middle period of digestion, and the relative position of the two nuclei varies very much even in the two products of one division. All stages from the round, rather dumpy crithidia to immensely long and very slender forms moving with great rapidity darting across the field in a flash are to be seen in the crop at the same time.

These different forms will be described in greater detail when the stained material comes to be considered. Division stages may also be seen, and these are often unequal.

Towards the end of digestion the type becomes much more uniform, and slender forms with little protoplasm and flagella hardly exceeding the length of the body seem to dominate to the exclusion almost entirely of other forms. These creatures very often have the kinetonucleus just anterior to and almost embedded in the trophonucleus. They seem at this stage, moreover, to have reached the limit of division as dividing figures were never found. It appears probable that death would now ultimately supervene unless injected into the blood of the vertebrate host.

Conjugation was carefully watched for, but no sign of it was found. It was expected to occur probably immediately after the first divisions in the leech or else possibly towards the close of the middle period of digestion.

The process of conjugation might of course occur at another point of the life-cycle, namely, at the time when the Trypanosome is injected from the alimentary tract of the leech into the circulation of the tortoise. If that were the case it would amply explain the elusive character of this process amongst Trypanosomes, as that is the one part in the cycle of a plasma dwelling form which it is almost impossible to investigate properly.

The question of the surviving of the Trypanosome in the leech has to be considered. It is a matter which is not easy to settle quite definitely, but I do not think that it occurs in this case. Leeches from uninfected hosts showed no parasites. I had a tortoise with only *Hæmogregarines* in its blood, and I never got Trypanosomes in leeches from this individual. As far as my observation goes (both as regards the investigation of the live creature and of sections) the parasite is never found outside the alimentary tract, and, while to be found in the intestine, is much more a crop parasite than, for instance, *T. raia* in *Pontobdella*.

How exactly the infection is transmitted to the tortoise in the act of sucking I do not know. But this much is pretty clear, the leech seems to suck by rhythmic contractions, that is to say, the suction is periodically inhibited.

I do not know if the Trypanosome, which is certainly slightly rheotropic, is sufficiently so to swim against the inward flow of blood during the suction time, but it might conceivably be able to do so in the intervals during which this is suspended. Further, the skin of the host must be pierced before suction begins, and it may be during this part of the process that the parasites are communicated to the vertebrate.

The habits of the *Glossiphonia* seem admirably adapted to the requirements of an intermediate host: its trick of wandering from one tortoise to another, its apparent conservatism in the choice of a host, and the relatively rapid digestion are all features favourable to the spreading of such a parasite as a Trypanosome.¹

III. OBSERVATIONS ON STAINED MATERIAL.

So much then for the observations upon the live material. The material for staining was treated in various ways; films were made and dried in air, then fixed in absolute alcohol—or they were exposed to osmic vapour and then allowed to

¹ See note on p. 693.

dry—or they were plunged while still wet into corrosive sublimate and acetic acid and treated by wet methods throughout. It is a matter of regret to me that I did not fix more material in this last way. The films were stained by various Romanowsky methods, or, in the case of wet films, in Heidenhain's iron hæmatoxylin or Ehrlich's hæmatoxylin.¹ The Trypanosome was also studied in section from the various organs.

The drying method followed by the Romanowsky stain gives excellent results with certain types of object, but may at times give misleading pictures, more especially with more massive creatures. It is therefore advisable, where possible, to control the results with material treated by wet fixation; osmic films are very valuable, but are open to the same objection in the matter of drying. The drying method seems to flatten and spread out certain types of organism; on the other hand, the wet fixation of blood-films equally certainly causes the parasite to shrink. This is very marked in certain Hæmogregarine phases. Fixation is generally a choice of errors, but by the combination of the dry and the wet method a very fairly accurate idea of the nuclear structure of the organism may be obtained.

Trypanosoma vittatæ (Pl. 16, figs. 1—7) in the blood of the vertebrate host shows dense protoplasm, markedly alveolar with longitudinal striations, corresponding to the myonemata so clearly visible in the live state. These are not always equally conspicuous. There is no doubt that the adult Trypanosome in the blood of the vertebrate is possessed of quite a definite outer sheath or periplast—this is clearly visible in crushed specimens. The myonemata appear to form part of this structure.

The protoplasm has a tendency to stain deeply, whatever the method used; granules and protoplasmic inclusions are never, in my experience, visible at this stage in the stained preparations. In the live state there are sometimes bright globules to be seen in the protoplasm; they are not very

¹ I am indebted to Prof. Minchin for advice on the handling of these wet preparations.

commonly present, and this occurrence is quite casual. They may possibly be of a fatty nature, and be dissolved out by the alcohol with which the slide is treated. Whatever their nature, they have no visible equivalent in the stained preparations. The membrane presents the usual structureless appearance, and takes up the stains very faintly and evenly. In the Romanowsky films, and also, though less frequently, in wet hæmatoxylin material, a line is to be seen on the membrane, just immediately inside the flagellum; it suggests a supporting or skeletal structure and takes up the plasma stain.

The flagellum runs at the edge of the membrane, and can be traced back very close to the kinetonucleus, but not as a rule actually into it. A minute granule¹ can occasionally be seen just at the root, but it is not by any means always visible. The kinetonucleus is rod-shaped; in Romanowsky preparations it shows as a more massive structure than in the wet films treated with Heidenhain. It is always surrounded by a slightly clearer area of protoplasm, but there is no sign of a definite vacuole in its neighbourhood as is described for some of the mammalian Trypanosomes.

In a dried film the Trypanosome is practically presented in one plane, and a certain amount of widening out occurs, this causes a disturbance of the internal structures to a greater or less extent. The form in question during its sojourn in the blood of the vertebrate is, relatively speaking, a massive creature, it is oval in cross-section, as can be seen in paraffin sections from the lung. It is therefore not particularly well adapted for the drying method. In the wet films the trophonucleus shows a large chromatic karyosome surrounded by a clear area, which, in turn, is bounded by a sharply-defined ring, which takes on a nearly black colour with iron hæmatoxylin (Pl. 16, figs. 1 and 2). I propose to use the word nuclear membrane for this outer ring. I merely do this as a matter of convenience; it is not at all clear how far it can be

¹ This corresponds apparently with the blepharoblast of Minchin, 'Q. J. M. Sci.', vol. 52, 1908.

regarded as a true nuclear membrane in the metazoan sense, and I do not wish the word to be taken as implying homology. I have never been able to see on hæmatoxylin preparations any strands passing from the karyosome to the membrane, although it is a very usual feature in this type of nucleus.

It is to be noted that the protoplasm shows no differentiation round the membrane of the nucleus, that is to say, that in the wet method films stained with hæmatoxylin there is no protoplasmic halo.

A slightly different appearance is presented in the dried films here (Pl. 16, figs. 3 and 4), there is a wide protoplasmic halo which always takes the blue stain of the Romanowsky combination much less deeply than the surrounding cell substance.

Approximately in the centre of this is the karyosome, which is composed of a blue staining substance underlying a reticulum of red staining chromatin. In some specimens strands seem to pass out from the karyosome—occasionally a pale (fig. 3) red ring surrounds the karyosome and strands may be seen passing between them. It is to be noted that this ring is well within the protoplasmic halo. My interpretation of the Romanowsky appearance is as follows:—The red ring corresponds to the membrane (in the Heidenhain preparations) which is generally destroyed in the drying.

What I have called the protoplasmic halo in the Romanowsky picture corresponds to the space between the karyosome and the membrane, but it is much widened by the pulling back of the protoplasm due to the general flattening of the whole organism.

As before noted small specimens are present, the origin of these (Pl. 16, figs. 5 and 6) is still obscure; they may either arise from the large forms by division or they may possibly be the young forms derived by infection from the lecch. I am inclined to consider the former explanation as the more probable. It is, however, impossible, to form any very reasonable opinion on the point except from the results of experimental inoculation.

These small forms have hyaline protoplasm staining faintly with the blue of the Romanowsky combination. The protoplasmic halo is not visible in these forms, and in the wet preparation the membrane or outer ring is always rather faint.

The kinetonucleus lies very close to the anterior end of the body which tapers very rapidly to a sharp point. The membrane is relatively wide and the free flagellum is long.

Division stages in the adult Trypanosome must be exceedingly rare, as although a very large number of well-infected films have been searched I have never come across any of the full-grown forms in this condition. Among the forms intermediate between the adult and the small specimens, however, individuals with two nuclei and dividing kinetonuclei are to be found; they are never numerous, and I can say nothing of the details of the process (Pl. 16, fig. 7).

As already indicated I can find no morphological grounds for dividing the adult organism as found in the blood of the tortoise into male, female, and indifferent. There is no evidence to suggest that the small specimens like those shown in Pl. 16, figs. 5 and 6, are males and the large females, and outside of this the difference among the specimens is very slight, and involves apparently only protoplasmic features. This, in itself, is, however, no argument against an ultimate sexual differentiation or conjugation in the intermediate host.

The material from the leeches, I am sorry to say, was mostly fixed by the dry method followed by alcohol, a good deal being fixed by the osmic vapour method to act as a control. The Trypanosome is also to be recognised on sections of the whole leech, but it is difficult to get a very clear and brilliant picture. However, the dry method is better adapted to the thin leaf-like shape of the Trypanosome in the intermediate host.

The detail of the very earliest changes in the Trypanosome upon being taken into the leech is difficult to get in the stained preparations as they occur before the leech has ceased feeding. However, they are sufficiently clear from

the live observations. As soon as the Trypanosome has rolled itself up and cast off its flagellum, the division of the nuclear elements begin, and it is at this stage that they are to be found in material from the crop. This early part of the work has mostly been made out from material from the *Limnatis* (figs. 8—12).

The two first divisions both of the trophonucleus and kinetonucleus follow quickly upon each other, the details of division being not so clear as in *T. raiaë*. Probably this is due to the slowness of the process in this last-mentioned Trypanosome, which in conjunction with the rich infections found in the intermediate host make the obtaining of a complete series a comparatively simple matter.

In the division of the trophonucleus of *T. vittatæ* there is a marked resemblance to what occurs in *T. raiaë*; in fact, the two processes are quite parallel. We have here also a nuclear division where no equatorial plate is formed, and where (figs. 9 and 10) no differentiation of the chromatin into chromosomes occurs. In both cases there is a well-marked spindle apparatus which appears to bring about the division of the chromatin with only a slight disturbance of its arrangement. So also in both instances the substance of the spindle apparatus is absorbed in the protoplasm and not enclosed in the reformed nuclei.

Fig. 9 shows a stage in this division process, and also fig. 10 in which the kinetonucleus has divided into four already, and both trophonuclei are in the act of undergoing the second division.

The kinetonucleus, however, shows a considerable difference from the conditions obtaining in *T. raiaë*.

In *T. raiaë* the kinetonucleus became greatly elongated, and then divided transversely, the products of division often remaining connected by a red staining band. In *T. vittatæ* the kinetonucleus when dividing splits through longitudinally. Sometimes the split starts at one end of the kinetonucleus, but takes a considerable time before being completed, and it may thus present a horseshoe shape. The

kinetonucleus generally precedes the trophonucleus in division, but this is not by any means invariable. I cannot find any sign of the centrosomal function described by França for the Trypanosome in *Hyla arborea*.¹ He describes a development of the Trypanosome resembling this early process in the leech, but in the case studied by him the kinetonucleus appears to enter into what he considers definite centrosomal relations with the trophonucleus at the time of division. This is quite absent in *T. vittatæ*.

As already stated the flagellum develops with no very close regard to the exact stage of the nuclear divisions. It grows out rapidly, and in the stained preparations seems to develop by direct outgrowth from the kinetonucleus. The complicated figures met with at a certain stage in the development of *T. raia* in *Pontobdella* are here entirely absent. The products of the divisions show a certain amount of variation as regards the state of their development at the time of being set free. Pl. 16, fig. 11, is a very typical example of the more rounded forms, fig. 12 is another, and a third is shown in fig. 12 *a*.

Twenty-four hours after feeding the parasites are all flagellate forms varying considerably in shape, but most of them already showing an undulating membrane, and the kinetonucleus has migrated as a general rule pretty far back though it is still in front of the trophonucleus (Pl. 16, figs. 11—13, 16, and 17). In the *Glossiphonia* this process is still quicker, and from this time on the infection in this leech begins to show the very wide range of forms so characteristic both of *T. vittatæ* and *T. raia*.

A review of Pls. 16 and 17, figs. 13—26, will give some idea of the various types present. Long slender forms, some with exceedingly elongated bodies and the kinetonuclei showing considerable variation in their relation to each other, typical Trypanosomes often somewhat broad in shape, and small dumpy forms with short flagella, are all present together at

¹ ("Recherches sur les Trypanosomes des Amphibiens," 'Arch. de L'Inst. Roy. de Bact. Camera Pestana,' T. i, Fasc. ii, 1907).

this middle stage of digestion. From such a range of forms it would be a simple matter to pick out creatures suggesting the morphological features which are considered characteristic of male and female individuals. Thus Pl. 17, figs. 23 and 25, might be regarded as respectively male and female; so also Pl. 17, figs. 24 and 26; but until the act of conjugation is observed I cannot see that there is evidence enough to make it clear that the quite obvious morphological difference is the expression of a sexual differentiation.

At this middle stage of digestion dividing individuals are of frequent occurrence. In *Trypanosoma vittatæ* unequal division at this stage is the rule rather than the exception. The process is figured in Pl. 17, figs. 18 and 19, and the different character of the products is obvious.

The new flagellum unquestionably grows out apart, and does not arise by splitting off from the old one. The splitting of the flagellum, as is well known, does occur in certain *Trypanosomes*; as, for instance, in *T. grayi* described by Prof. Minchin,¹ and it is of interest to find two such very different methods holding good in the group.

The new flagellum in *T. vittatæ* will start growing out from the kinetonucleus before it is obviously divided, though it is generally enlarged. A review of Pls. 16 and 17, figs. 16—20, will demonstrate this question pretty clearly.

As time goes on the nature of the infection undergoes a very marked change; the predominating type of *Trypanosome* is now found to be a slender rather short form with the flagellum extending only to a short distance beyond the end of the body. The undulating membrane is very narrow. The tropho- and kinetonuclei have entered into close relations with each other, and generally the kinetonucleus is just anterior though closely applied to the trophonucleus. Specimens are found with the kinetonucleus posterior, but these are few in number. The gradual predominance of this type to the exclusion of practically every other can be

¹ "Trypanosomes in Tsetse flies and other Diptera," 'Q. J. Micr. Sci.,' vol. 52, 1908.

followed very clearly in different infections. Thus Pl. 17, figs. 31—33, came from a quite empty leech, and only this type with very slight variations was present. Pl. 17, figs. 18—27, come from a leech in the middle period of digestion, and this final type is just beginning to appear in isolated specimens. In another leech a little further advanced it is more numerous than any other, but not exclusively in possession of the field.

I have never seen division in this type which appears at the end of digestion. I am inclined to think, although there is no actual evidence, that these forms die off unless injected into the blood of the vertebrate.¹

Exactly what happens to the forms which disappear during the course of digestion is not very clear. The broad forms, however, appear to give rise to such forms as Pl. 17, figs. 29—33, simply by division and direct development. The case of the very long slender forms (Pl. 17, figs. 25, 26, and 28) is more difficult, and the material at my disposal does not throw much light on the question.

IV. GENERAL REMARKS AND CONCLUSIONS.

The life-history of *Trypanosoma vittatæ* shows a close resemblance to that of *T. raia*. Some quite recent observations upon this last mentioned Trypanosome made in November, 1908, at the Millport Marine Station with *Pontobdella* which I had reared from the egg, have amply confirmed Brumpt's brief sketch of the early stages of the life cycle.²

Moreover, skate's blood, which contained the Trypanosome kept upon sealed slides has shown that here also the Trypanosome discards its flagellum and undergoes a number of divisions. The process is similar to that of *T. vittatæ*, but the rounded non-motile stage seems very much more persistent. In the *Pontobdella* motile flagellate stages do not

¹ It is very difficult to be certain that division never takes place in this form, but I have never seen any sign of it.

² 'C. R. Soc. Biol.,' ix, 1906.

begin to appear for four to six days, although the flagellum itself may be present for some time, apparently more than a day, before it becomes motile. The earliest divisions of the rounded Trypanosome, instead of following each other immediately as in *T. vittatæ*, are here at intervals of at least twenty-four hours. The early stages of development of the flagellum are also very slow.

This process by which the Trypanosome rounds itself off and after a number of divisions produces a definite Crithidial flagellate, appears to me to be more widespread among Trypanosomes of a certain type, notably those from cold-blooded hosts, than it has hitherto been considered.

França observed an analogous process some time ago in the Trypanosome of *Hyla arborea*, also in *T. granulorum*.¹ Brumpt describes it for this Trypanosome also in the leech *Hemiclepsis*, and Dutton, Todd, and Tobey² have seen it in *Trypanosoma loricatum*.

Another point of interest in the life cycle of *T. vittatæ* is the very marked development of a uniform slender type of parasite at the end of digestion. In *T. raia* this also occurs, though here some rounded forms seem often to persist. A somewhat similar development occurs in *T. grayi*,³ where the last stage of the infection shows, however, two slender forms, one of which, a *Herpetomonas*-form, encysts in the proctodæum of the *Glossina*, while the other does not encyst, and seems probably to be destined to transmit the infection by inoculation into a vertebrate host.

I need not emphasise the absence of evidence of conjugation. This process if it occurs seems particularly difficult to observe in Trypanosomes, and no quite satisfactory account of it has yet been given for this group of flagellates.

It is a point of only slight interest, but it is curious to note to what an extent the infection is capable of persisting in

¹ 'Bull. Soc. Portug. Sc. Nat.,' vol. i, p. 3, Dec., 1907.

² 'Ann. Trop. Med. and Parasit.,' vol. i, No. 3, 1907. (I have not seen the original memoir.)

³ Minchin, loc. cit.

the *Limnatis*, which is apparently not a true transmitting host.

The work here recorded was done partly at the Government Museum in Colombo, Ceylon, and partly in the Zoological Laboratory in the University of Glasgow. I am much indebted to Prof. J. Graham Kerr for kind suggestions during the course of the work.

GLASGOW; December, 1908.

NOTE.—It may be objected that no absolute proof has been adduced that the form in the *Glossiphonia* is *T. vittatæ*. Absolute proof was not possible from the nature of the conditions, but the evidence seems to me to be very strongly in favour of the identity of the parasites. The correspondence of the early stages in *Glossiphonia* with those in *Limnatis* where experimental feeding could be carried out, the final stage in the *Glossiphonia* at the end of digestion being a slender flagellate and not a rounded-off organism as occurs in the flagellate of non-bloodsucking insects, and the absence of the parasite in the leeches from the tortoise where no *Trypanosomes* could be found all seem to me to point to the stages in *Glossiphonia* being true developmental stages in the life cycle of *T. vittatæ*.

I may add that no flagellates were ever found in the land leech or in *Ozobranchus* or in *Limnatis*. *Ozobranchus*, of which a very large number were examined, lives in exactly the same habitat as *Glossiphonia* but is parasitic on *Nicoria trijuga* instead of *Euryda vittata*. *Nicoria* never showed a *Trypanosome*.

EXPLANATION OF PLATES 16 AND 17.

All figures were drawn with the Abbé camera under a 2 mm. Zeiss apochromatic immersion objective. A No. 12 compensating ocular and tube length of 250 mm., giving a magnification of approximately 3600 diameters. This has been reduced by the lithographer to approximately 2400 diameters.

Figs. 1-7.—*Trypanosoma vittatæ* from the blood of *Emyda vittata*.

Fig. 1.—Trypanosome from blood of tortoise stained with Heidenhain's iron hæmatoxylin after fixation with corrosive and acetic, wet method throughout.

Fig. 2.—As above, showing a characteristic attitude.

Fig. 3.—Dried Giemsa film of Trypanosome showing protoplasmic halo and red ring round karyosome.

Fig. 4.—Trypanosome as above, showing myonemata and line along undulating membrane.

Figs. 5 and 6.—Small specimens from blood of tortoise.

Fig. 7.—Dividing stage from blood of tortoise.

Figs. 8-12A.—Early stages in crop of leech. These are from the water leech, *Limnatis granulosa*.

Fig. 8.—Division stage; the four new flagella are already developed; kinetonuclei are dividing by longitudinal splitting.

Fig. 9.—Division stage showing nuclear spindle.

Fig. 10.—Second division occurring before the completion of the first as regards the protoplasm. Both kinetonuclei in act of division, only two flagella so far developed.

Fig. 11.—Rounded flagellate—the product of the divisions of the rounded off Trypanosome.

Fig. 12.—Early flagellate stage; note the lengthening of the body.

Fig. 12A.—Another early flagellate stage.

All the remaining figures are from the *Glossiphonia*, with the exception of 16 and 17.

Figs. 13-15.—Osmic fixed film from leech just about the beginning of the middle stage of digestion.

Fig. 13.—Flagellate stage showing elongated body.

Fig. 14.—Flagellate, with broad posterior end.

Fig. 15.—Very long flagellate kintonucleus at same level as trophonucleus.

Figs. 16, 17.—Early flagellate stages from horse leech to show secondary increase in size and preparation for division. Note the condition of the flagella showing outgrowth from the kintonucleus.

Figs. 18-27 from the *Glossiphonia* at middle stage of digestion.

Fig. 18.—Division stage. Note relative position of the kintonuclei and the condition of the flagella. The unequal character of the division is obvious.

Fig. 19.—Another division stage. The features are much as in Fig. 18.

Fig. 20.—Early division stage to show condition of kintonucleus and flagella.

Fig. 21.—Trypaniform individual with broad posterior end and many red-staining granules in the protoplasm.

Fig. 22.—Small broad form.

Figs. 23 and 24.—Short, rather broad, trypaniform individuals.

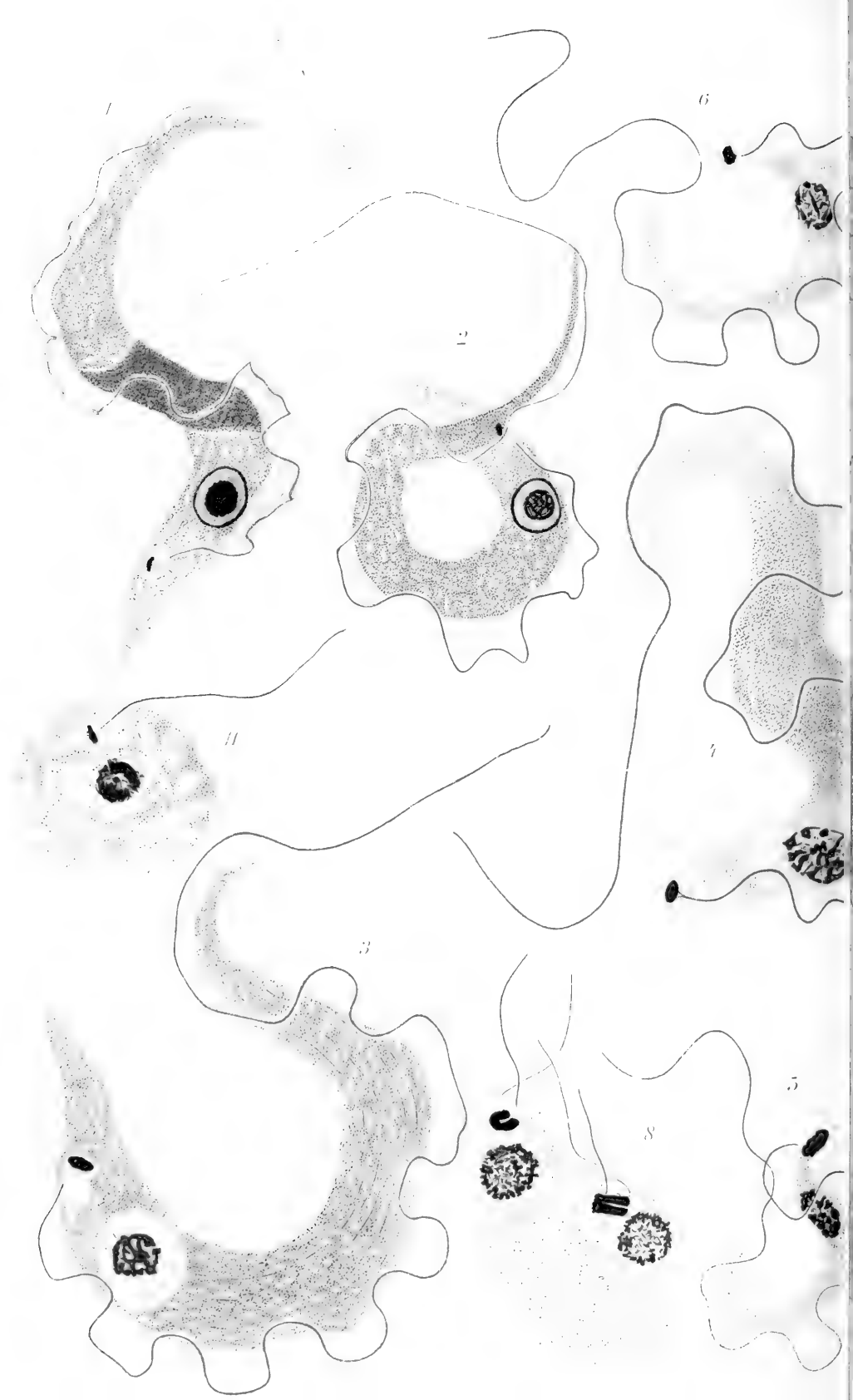
Figs. 25 and 26.—Long slender forms.

Fig. 27.—Isolated forms of this type are just appearing in this leech which is at the middle stage of digestion. This is very like the final type developed at the close of digestion.

Fig. 28.—Very long slender form.

Figs. 29 and 30.—From a leech whose digestion is still more advanced. Note the difference in the type of the organism.

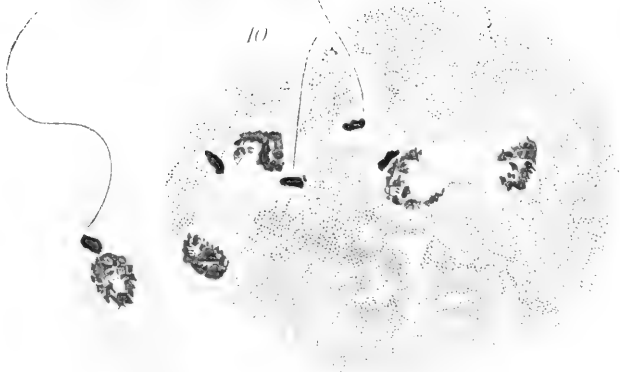
Figs. 31-36.—Flagellates from leech at end of digestion. this type alone is present with very few exceptions.



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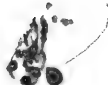
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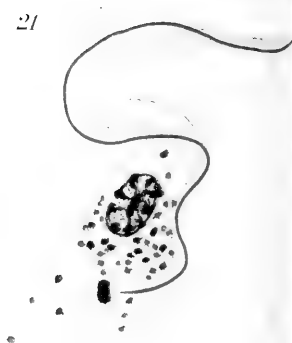
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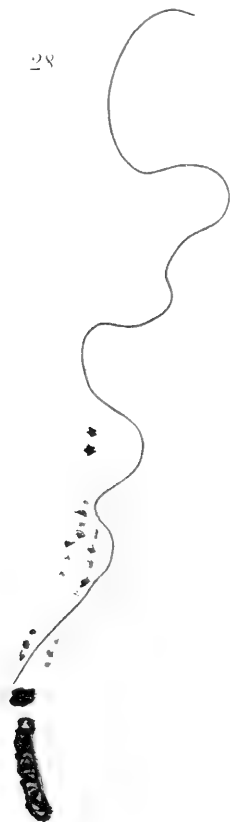
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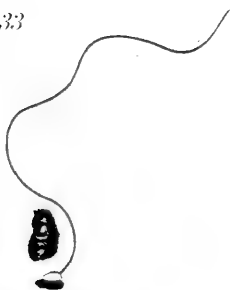
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The Entry of Zootxanthellæ into the Ovum of Millepora, and some Particulars concerning the Medusæ.

By

Joseph Mangan, M.A., A.R.C.Sc.I.,

Honorary Research Fellow of the University of Manchester.

With Plate 18.

During the present winter Prof. S. J. Hickson kindly gave me an opportunity of examining the material from Jamaica in which he discovered the female Medusæ of Millepora ('99). The results of my inquiry are mainly concerned with the manner in which the symbiotic zootxanthellæ, found to exist in the free ova of that genus, infect the germ cells. I also wish to record the occurrence of free male medusæ amongst the free female medusæ collected in the above locality, and to make some remarks upon the oogenesis.

THE INFECTION OF THE OVUM BY ZOOXANTHELLÆ.

In the earliest stages of the female medusa that came under observation the germ cells are arranged around the manubrium of the medusa, as a single layer, in most instances, in dome-shaped fashion. Each cell has well-defined boundaries, and possesses a large nucleus with intensely-staining chromatin nucleolus (fig. 1). The substance of the manubrium is vacuolated and scattered; in it lie numerous zootxanthellæ. At the points where ova arise it would appear as if a gradual dissolution of the cell membrane took place between the cell destined to become the ovum and

the cells in contact with it. The nuclei of these sister-cells must undergo dissolution before such a plasmodium is formed, for I have never observed them in the growing ovum, which, moreover, is often bordered by cells in which the nucleus is but faintly discernible (fig. 2). The ovum continues its growth, in this manner at the expense of its sister cells, until at or about the time of liberation of the medusæ the last are incorporated; and I have observed that one or two of their nuclei, at this stage, have in some cases persisted in the cytoplasm of the egg (figs. 3, 8, 9, 10). Finally the ova encroach upon the vacuolated substance of the manubrium until it is reduced to very small proportions (fig. 12), when they are set free.

In the material examined it was clear that the vacuolated substance of the ova in the free medusæ, in all cases, contained numerous zooxanthellæ; but they were equally absent from the ova of the numerous unliberated medusæ that had been inspected. However, after a long search some half a dozen medusæ were found which, I think, afforded conclusive evidence that zooxanthellæ pass in numbers from the manubrium into the ovum, and that this invasion may commence at a period prior to the liberation of the medusæ.

The ovum, until it has attained a considerable size, is sharply divided off from the vacuolated substance of the manubrium. Eventually a period is reached when its compact cytoplasm becomes continuous with the central meshwork (fig. 8). This stage could be commonly observed, and zooxanthellæ be seen round the margin of the egg, but never in its compact substance. Sometimes the egg cytoplasm displayed small incipient vacuoles.

The subsequent period, during which vacuolisation of the cytoplasm of the ovum occurs, and during which the zooxanthellæ are incorporated, could be found only on one piece of *Millepora*, where half a dozen medusæ had reached this stage (fig. 9). Their ova showed a variable amount of vacuolisation, which, so far as I could make out, is in great part inaugurated at the inner borders of the egg substance; as

if in some manner the vacuolated portion of the manubrium with its contained zooxanthellæ were drawn in.

That the medusæ are liberated at this stage can be concluded from their mature appearance within the ampullæ, and from the fact that one free medusa (fig. 10) was met with which exhibited an essentially similar structure.

The other free medusæ examined, possessed ova, more completely vacuolated, and with more numerous zooxanthellæ. The egg-cells showed various phases of an encroachment upon the manubrial substance, which eventually was almost entirely reduced (fig. 12), the ova at the same time becoming rounded off and similar to the extruded ova that were examined.

During all this period the zooxanthellæ exhibit their normal appearance, and it can be observed that they divide fairly frequently within the ovum. These cells have been figured by Moseley ('81) who was able to examine fresh material. He remarks that they closely resembled those of other Hydroids. They contained irregular granules of a bright gamboge-yellow colour, the cell-contents frequently dividing into two, and sometimes, more rarely, into four. In the older portions of the colony the pigment was of dark-brown hue. I show their structure as displayed in stained specimens (fig. 18). The spherical nucleus exhibited a mass of closely-packed chromatin granules. A pyrenoid was always present, the clear space around which, in most cases, gave the reaction for starch. The pigment-bearing granules varied in number and size, did not always stain to the same degree, and in some cases had a little starch associated with them. The cell membrane did not respond to cellulose tests. I observed in a few cases division of a cell into four. Their average diameter was somewhat over 9μ .

That zooxanthellæ pass into the ovum from the parental tissues appears to be undoubtedly the case from the foregoing evidence; but the part these play in the future economy of the animal remains to be solved. It may be that their enclosure is more accidental than physiologically necessary, for

the bulk of the foreign cells in the canals and tissues of the colony may come from the surrounding water at a subsequent period. An apparent increase in the substance of a mature ovum (see below) might be an indication of their activity. At all events, there is suggested, an approach to a more complete symbiotic union than that which exists in *Convoluta roscoffensis*, for instance, where it has been definitely shown ('07) that infection takes place after the animal is hatched, for there the animal undoubtedly plays, in the long run, the part of a parasite with respect to the alga, as under no known conditions were algæ found to escape alive from the body of that turbellarian.

THE MEDUSÆ.

Male medusæ of *Millepora* have up to the present been found only in material from Torres Straits and from Funafuti ('91), and none of these had been extruded from the colony, though some lay free in the ampullæ. However, along with the liberated female medusæ from Jamaica were two free male medusæ, one of which is figured (fig. 17). The most noteworthy feature of the anatomy at this stage is that the margins of the umbrella are furnished with some batteries of nematocysts. They are the largest of the smaller of the two varieties of stinging cells peculiar to *Millepora*. The manubrium is practically devoid of zooxanthellæ.

The anatomy of the female medusa has been figured and described by Hickson ('99), and is exhibited to some extent in figs. 8 and 9. In the later stages of the medusæ, within the ampullæ, the substance of the umbrella becomes thinned out centrally into an excessively fine membrane (fig. 9).

The structure of the liberated female medusa was exhibited fairly well in a few cases. A portion of a medusa, shortly after liberation, is shown in transverse section (fig. 10). A stage is also figured in which the eggs are evidently completely developed and ready for liberation (fig. 12), and, at

this period, it will be seen that the manubrium has been reduced to very small proportions. An enteric cavity persists, but no mouth. The margins of the umbrella contain four or five batteries of the largest nematocysts of the smaller variety, and also numbers of the smallest forms. The swellings carrying these batteries are the reduced tentacles figured by Duerden ('99) from living specimens in his aquarium.

In the majority of cases the free ova did not differ in their structure from those of the last-mentioned stage (fig. 12), their substance being uniformly vacuolated. However, two specimens were exceptional, showing numerous islands of compact cytoplasm in the alveolar ground-mass. One of these contained what, I think, may be the cleavage-nucleus in process of division (fig. 15). Its islands were free from chromatin. The other had some half a dozen chromatic bodies in as many of the islands (fig. 16), and probably represented a later period in the history of the egg prior to segmentation. As both were of more than average diameter, the presence of numerous areas of compact substance suggested that an accession of material had in some way taken place. Perhaps, as mentioned above, this, if the case, may be brought about by the activity of the included zooxanthellæ.

CONCERNING OOGENESIS.

A. Growth of the oocyte.

The origin of the germ-cells could not be traced out owing to the peculiar fact that the material containing the earlier stages of the female medusæ always exhibited individuals which were at approximately the same period of their development. No doubt the germ-cells, as in the case of the male elements ('91), move into the dactylozooids and gasterozooids, which subsequently undergo modification into medusæ. However, they were always found in the above material, as a single, or

in parts double, layer of superficial cells, upon the dome shaped, or at times conical, manubrium of a completely formed medusa.

The cells of this layer vary but little in size, and contain a proportionately large nucleus (fig. 1). The chromatin forms a close reticulum, and is mostly concentrated at the nodes. There is a single deeply staining chromatin-nucleolus.

In the material containing the subsequent stages the ova were practically all approaching the end of their growth period, or in some later phase. The smallest of the two or three exceptional instances (fig. 2) apparently exhibited the oocyte as a plasmodium resulting from fusion of the cytoplasm of several oogonia, the nucleus of the oocyte, however, alone persisting. Some oogonial cells (fig. 2) were in process of fusion with the ovum, and in others the nucleus was becoming indistinct. The germinal vesicle had increased in size, its chromatin now forming open branching strands. The nucleolus was present. In the next smallest (fig. 3) the nucleus was larger, showing to better advantage the branching chromatin strands; the single nucleolus persisted unchanged.

In what I took to be the succeeding stage to the foregoing two cases the nucleolus was never present in the germinal vesicle; which latter body seemed to have reached its limit of expansion. The elongate chromatin strands (fig. 4) were fairly numerous, lying mostly in the peripheral portion of the nucleus, and often exhibiting a ragged outline.

In the greater proportion of growing ova examined the chromatin strands were less conspicuous, fewer in number, and lay in contact with the nuclear membrane, or but a little distance from it (fig. 5). However, they could decidedly be traced into centrally situated, intermingling, achromatic strands, their staining capacity undergoing a gradual diminution towards the interior. In many ova the germinal vesicle was at first sight homogeneous and devoid of chromatin (fig. 6). Though in some such cases the most careful research re-

vealed no chromatin, yet as a rule minute feebly staining strands could be found projecting here and there from the nuclear membrane. Practically always a more or less distinct achromatic reticulum was to be made out in these clear nuclei.

The next phase exhibited by the nucleus was associated with the termination of growth on the part of the ovum. The chromatin, absent from the last of the preceding stages, can again be discerned, and reappears as minute feebly staining granules, which are often clearly at the nodes of an achromatic reticulum (figs. 8, 9). In ova of free medusæ these granules become deeply stainable (fig. 10).

The oogonia that do not develop into ova are practically all absorbed into the substance of the ovum; before this takes place their nuclei fade away (fig. 2), and lose their identity in a manner which I could not determine. Though in the later stages oogonial cells not in process of fusion with the oocyte may exhibit a homogeneous nucleus (fig. 7), yet the nucleolus in these stains deeply to the last. In three ova, belonging to just liberated medusæ, I have observed the nucleus of one of these cells persisting in the cytoplasm (fig. 10). The nucleolus appeared to be broken down into smaller granules. A few similar nuclei could be observed in the vacuoles of the manubrium (fig. 10).

The early stages in the development of the Cœlenterate egg have formed the subject of a memoir by Trinci ('06), wherein he describes several types, and gives an exhaustive discussion of the work that has been done in this direction up to the present. Prior to the growth of the egg it would seem that many cœlenterates reveal a synapsis stage, that is to say, the reticulate nucleus is converted into a closely coiled spireme, which Maas ('97) found resulted from the union of distinctly double chromosomes in the case of *Periphylla* and *Atolla*. The chromatin thread then breaks up, as growth commences, into numerous strands, which may practically disappear, except here and there alongside the nuclear membrane (*Phialidium*), or which may persist

(*Tiarella*). In the former instance the chromatin shows an increase towards the approach of maturation. The nucleolus is single in the beginning in all cases, but in one type this body may subsequently fragment and undergo changes in staining properties, losing its affinity for basic dyes. Whether single or multiple the nucleolus vanishes before maturation of the egg. In very many cases chromatic bodies make their appearance in the cytoplasm close to the nuclear membrane, suggesting, particularly when the vesicle becomes achromatic, that chromatin is cast out of the nucleus. Bearing the foregoing facts in mind, we may briefly review the phenomena in *Millepora*.

A synopsis stage such as figured by Trinci ('06) for *Tiarella* and *Phialidium* was not observed; however, the subsequent appearance of branching and solitary chromatin strands in the expanding nucleus, found a close parallel in *Millepora*.

In *Millepora* the chromatic strands gradually lose their staining capacity until the nucleus is practically, if indeed not absolutely, achromatic. About the time when the ovum has absorbed all its sister cells, the chromatin reappears as minute, diffuse granules.

The single nucleolus vanishes at an early period in the growth of the oocyte.

There is nothing suggestive of an expulsion of chromatin into the cytoplasm during the growth period, though of course an extrusion of non-chromatic substance could go on undetected. The achromatic phase of the vesicle is only temporary.

B. Subsequent phenomena.

Until the cytoplasm of the ovum is completely vacuolated its nucleus remains situated centrally, exhibiting deeply staining granules, uniformly throughout its substance, at the nodes of a fine achromatic reticulum (fig. 10). A nucleus which had begun to assume an oval shape, and was moving

to the surface of the egg, was of a like structure, but some of its chromatin granules were of increased size, and as a whole seemed to have contracted away from the nuclear surface for some little distance (fig. 11). I could distinguish no nuclear membrane in this case.

In ova that were ready to be spawned, or had actually been so, the germinal vesicle, now without any semblance of spherical form, and lacking a definite membrane, though contrasting sharply with the cytoplasm, lay close to the exterior, beneath a slight depression on the surface of the egg. It exhibited some remarkable features. The granules of chromatin were confined to certain areas and in many cases were of large size and few in number (figs. 12, 13). In several instances there were found, scattered about in the nucleus, in half-a-dozen or so groups, evidently without any regular arrangement, deeply-staining fragments which had a tendency to exhibit a form suggestive of minute tetrads (fig. 14). No achromatic structures were revealed, but, as at other times, the necessity for a supply of suitably fixed material, on which to confirm the appearances presented, was felt. In several ova deeply-staining granules, similar to those in the nucleus, were observed here and there in the cytoplasm, suggesting strongly that these latter may be cast out to some extent.

Ova that had been liberated possessed a rather uniformly-vacuolated structure, but in a certain instance compact cytoplasmic areas lay scattered throughout the vacuolated substances. This ovum had not the peripheral nucleus recorded of the previous stage, but beneath a slight depression in the surface of the egg, just where one would expect to find it, there was a patch of the more compact protoplasm containing two groups of numerous chromosomes (fig. 15). No traces of a spindle could be seen between them; the axis of such, if it existed, would be tangential and not radial. Another ovum had a similar general structure, but contained some half-a-dozen chromatic bodies in as many of the islands (fig. 16). Five of these were compact and reticulate in structure, the

sixth being formed of a number of open intermingling strands.

The limited number of free ova examined, and the possibility of their having been subjected to abnormal conditions, owing to such causes as the concentration of the water in the aquarium, precludes a rigorous discussion of the foregoing facts. However, it is interesting to note that the changes recorded are not at all remotely paralleled in certain forms such as *Distichopora* ('94) and *Pennaria* ('04), where a remarkable behaviour of the nucleus during the maturation period has been recorded. In *Millepora* we have, as in those cases, a migration of the nucleus to the periphery with a dissolution of the nuclear membrane, and an apparent casting out of chromatin into the cytoplasm until hardly any remains. In those forms the nucleus may lose its identity completely, which I have not observed in the limited specimens at my disposal. I think it possible that the maturation phenomena are in a like manner obscured in *Millepora*. The stage represented in figure fifteen I take to be the first division of the cleavage-nucleus, while figure sixteen presents a further stage in which several nuclear divisions have taken place in the as yet unsegmented egg.

The above-mentioned anomalous behaviour of cœlenterate ova at maturation is discussed fully by Hickson ('94) and more recently by Hargitt ('04, '06). A paper by Lillie ('06) bearing on differentiation in the egg, normal and artificial, may be found of particular interest in this connection.

THE ZOOLOGICAL LABORATORY,

THE UNIVERSITY OF MANCHESTER.

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EXPLANATION OF PLATE 18,

Illustrating Mr. Joseph Mangan's paper on "The Entry of Zooxanthellæ into the Ovum of *Millepora*, and some particulars concerning the Medusæ."

FIG. 1.—Oogonial cell from the medusa of *Millepora* at a period prior to the formation of ova. $\times 1600$.

FIG. 2.—An early stage in the growth of an ovum. The nuclei of the surrounding oogonial cells undergo dissolution, fusion with the ovum taking place subsequently. $\times 500$.

FIG. 3.—A somewhat larger ovum. The nucleolus still persists; the chromatin reticulum has been resolved into many open and branching strands. $\times 500$.

FIG. 4.—The nucleus of an ovum which had absorbed the majority of its associated oogonial cells. Peripherally are numerous chromatin strands. The nucleolus has disappeared. $\times 500$.

FIG. 5.—The nucleus of an ovum of the same period, with fewer chromatin strands and with central achromatic reticulum. $\times 500$.

FIG. 6.—A similar nucleus from which chromatin is practically absent. $\times 500$.

FIG. 7.—An oogonial cell persisting at this period with homogeneous germinal vesicle and sharply-defined nucleolus. $\times 500$.

FIG. 8.—Transverse section through a female medusa prior to liberation. The boundary of the ovum is ill-defined, its substance free from vacuoles and from zooxanthellæ. Chromatin is present as diffused, faintly staining, granules, which form the nodes of an achromatic network. $\times 200$.

FIG. 9.—Vertical section through an ampulla containing a female medusa. The margins of the umbrella are connected by an excessively fine membrane. The ova are undergoing a process of vacuolisation, and zooxanthellæ are being admitted. The nucleus, shown in the ovum to the left, contains diffused chromatin granules forming the nodes of an achromatic network. $\times 160$.

FIG. 10.—Transverse section through a free female medusa. The ovum is only slightly vacuolated, and contains but few zooxanthellæ; most other ova of free medusæ were much more advanced in these respects. Below the nucleus there is seen in the egg cytoplasm the degenerate nucleus of an oogonial cell, and to the left of the ovum two such bodies are in the substance of the manubrium. The chromatin granules of the ovum nucleus stain quite deeply at this stage. $\times 200$.

FIG. 11.—An ovum nucleus from a completely vacuolated egg. It had lost the spherical contour of the preceding stages, and was situated rather peripherally. The deeply staining chromatin granules form the nodes of an achromatic reticulum, and are absent from a small superficial area of the vesicle. $\times 500$.

FIG. 12.—A transverse section through a liberated female medusa. The three ova are completely vacuolated, contain numerous zooxanthellæ, and have their nuclei situated at the surface. The germinal vesicle has lost its spherical shape; its chromatin granules, some of which are of large size, are collected to a great extent in one portion of

the nucleus. The substance of the manubrium has been for the greater part, encroached upon by the ova. $\times 120$.

FIG. 13.—A nucleus from an ovum of preceding figure. $\times 500$.

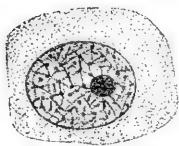
FIG. 14.—The nucleus of an extruded ovum, with a portion of the egg cytoplasm showing four zooxanthellæ. In the central portion of the nucleus exists an aggregation of variously shaped chromatic bodies. $\times 500$.

FIG. 15.—Portion of an extruded ovum, showing two chromosome groups in a region of more compact cytoplasm. $\times 500$.

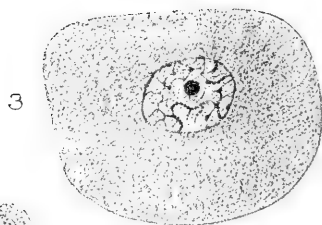
FIG. 16.—An unusually large extruded ovum, with islands of unvacuolated cytoplasm; two of these containing compact chromatic bodies, and a third more open chromatic strands. $\times 200$.

FIG. 17.—A liberated male medusa. The manubrium is surrounded by countless minute spermatids. On the umbrella are slight swellings bearing some large nematocysts. $\times 120$.

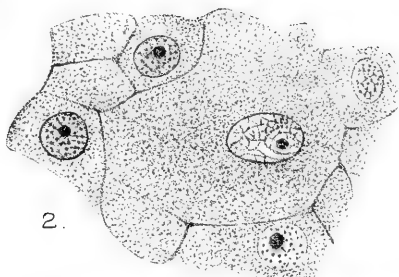
FIG. 18.—A zooxanthella, showing nucleus, pyrenoid, granules, and cell membrane. $\times 2000$.



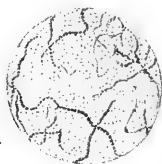
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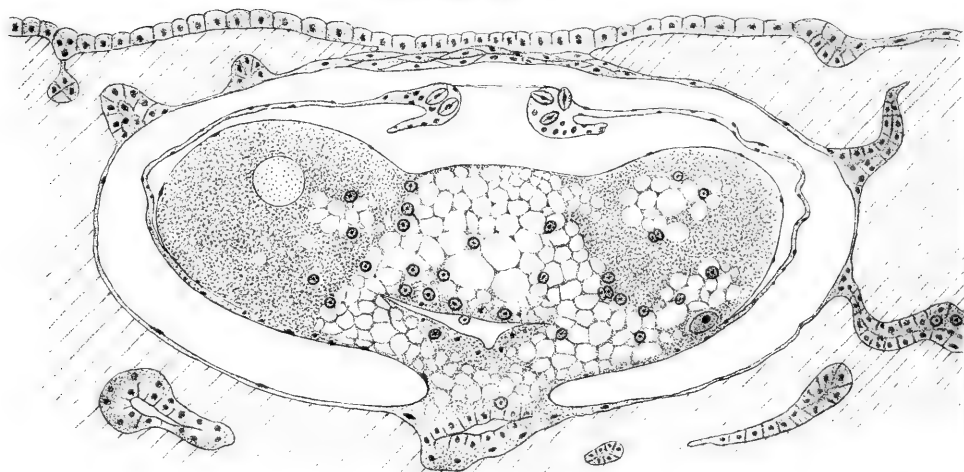
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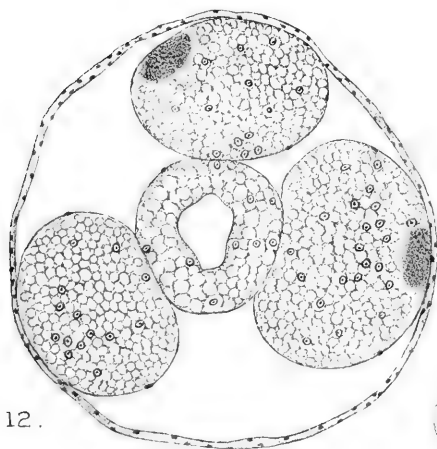
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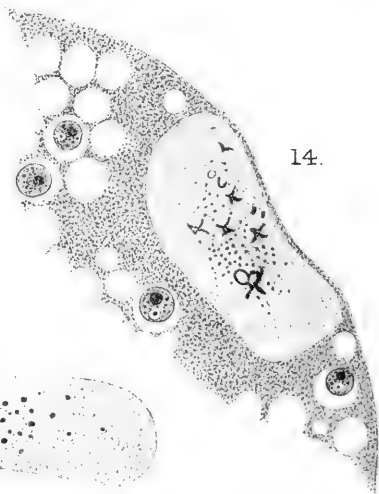
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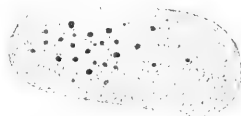
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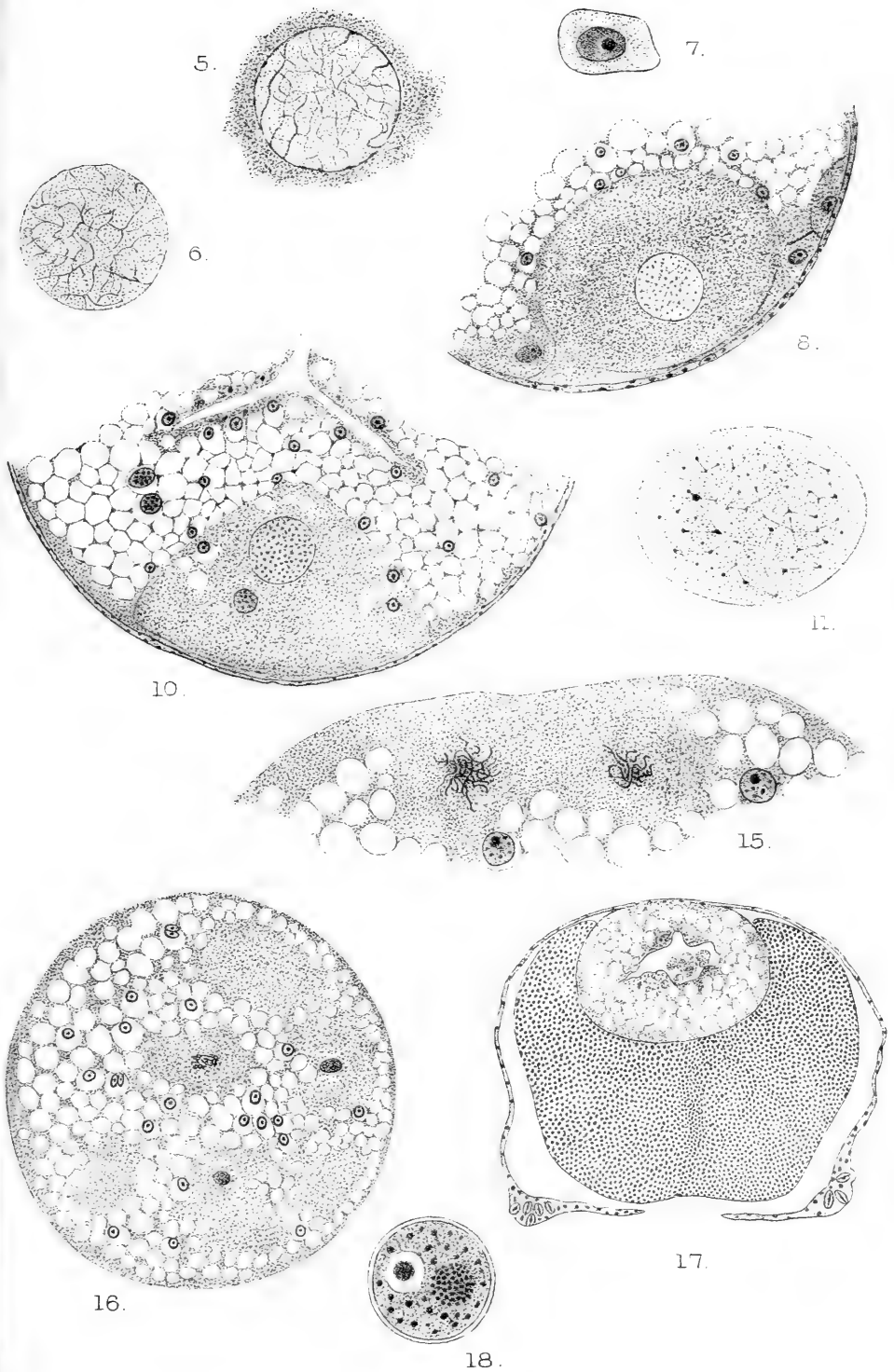
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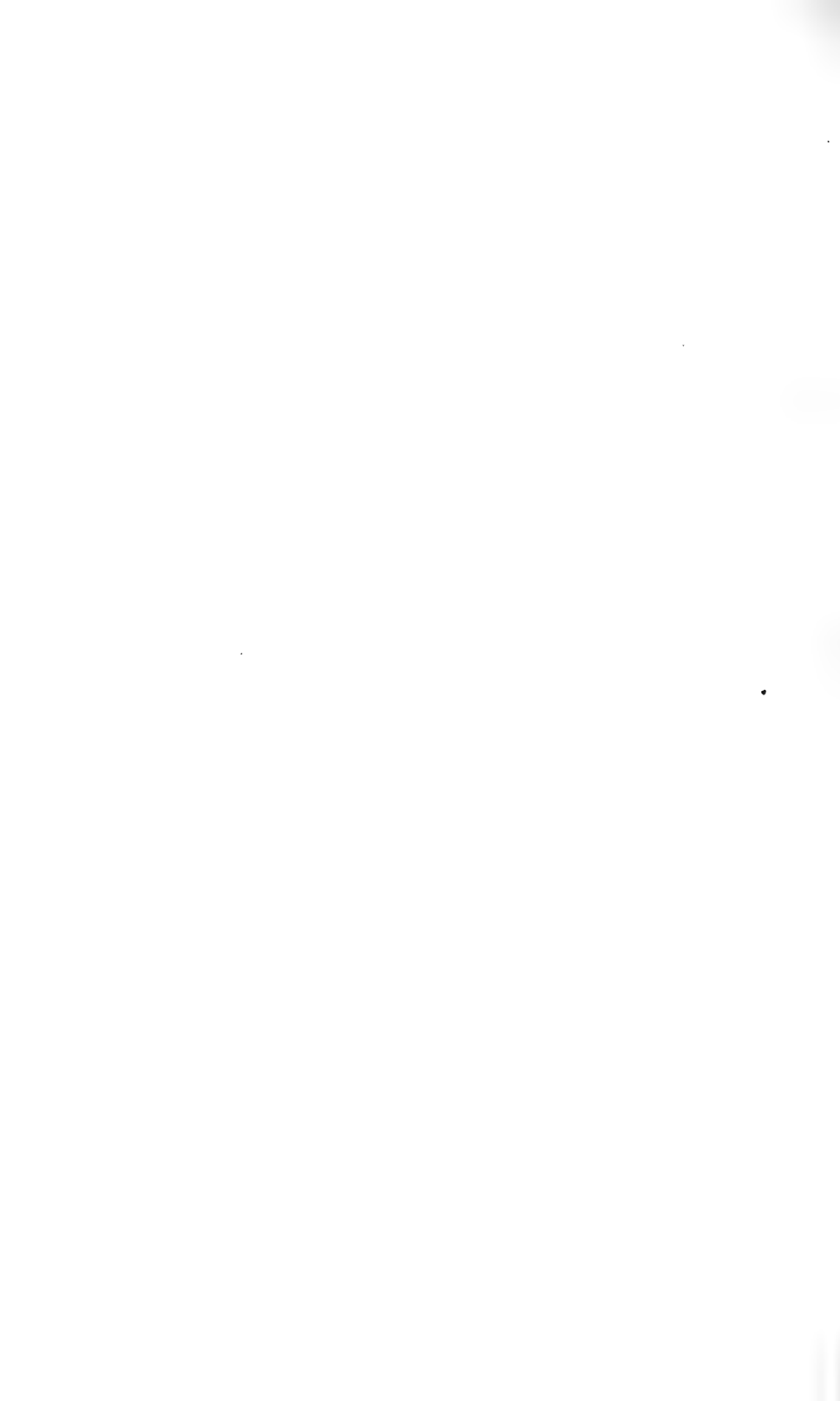


14.



13.





Physiological Degeneration and Death in *Entamœba ranarum*.

By

C. Clifford Dobell,

Fellow of Trinity College, Cambridge; Balfour Student
in the University.

With 5 Text-figures.

IN the following pages, I wish to call attention to some remarkable events which I have observed in the life-history of *Entamœba ranarum* Grassi, an organism which I have been studying for some time, and whose life-cycle—so far as I had succeeded in following it—I have already described in a previous paper (2). I will divide my remarks into two sections—I, a description of the phenomena actually observed, and II, a discussion of their significance.

I.

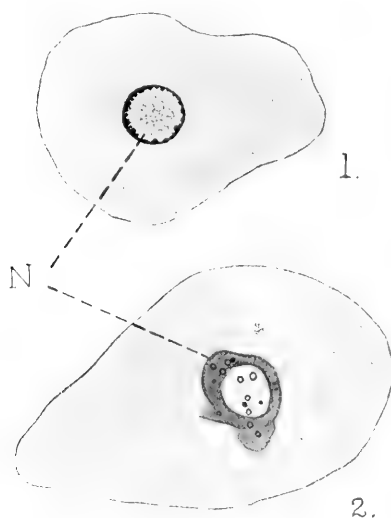
I have given elsewhere (2) a detailed description of *E. ranarum*, but I must here briefly refer to the structure of an ordinary individual once more. A typical amœba, taken from the rectum of a frog or toad, measures—roughly speaking—20–30 μ in diameter, and has a nucleus whose diameter is about 6 μ . For comparison with the forms I am about to describe, I have given a figure of an ordinary organism (text-fig. A, 1), showing the structure of the nucleus. The latter has most of its chromatin in the form of granules arranged peripherally, so that it has an annular appearance in optical section.

Now I occasionally found forms which differ from these

ordinary forms in a most striking manner. They are usually of larger size ($40-50\mu$) and possess a much modified nucleus (text-fig. A, 2). Although this modification varies—both as regards its type and extent—in different individuals, it is nevertheless always characterised by two features—enlargement and peripheral thickening (c f. text-fig. A, 2). Very often, the edge of the nucleus is thrown into folds (text-figs. B, C, 1).

As will readily be seen from the figures, these modified

TEXT-FIG. A.



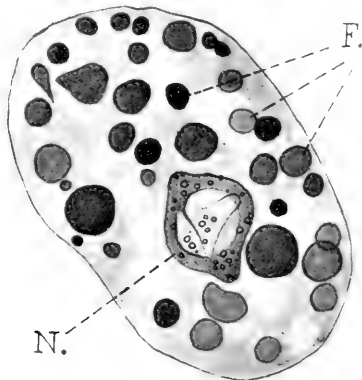
Entamoeba ranarum. Drawings (to scale) showing the structure of the nucleus (N) in an ordinary individual (1) and in one undergoing degeneration (2), in optical section. (The structure of the cytoplasm is not shown.)

forms are very striking. For some time I was unable to determine their origin, and their proper place in the life-cycle of the amoeba; but I have now been able to prove that these unusual forms are undergoing a process of degeneration, which finally results in death. At first I obtained various isolated stages in the process in animals from different hosts, at different times, but I have now succeeded in following out

the whole process in the amœbæ from two toads which I recently examined (January, 1909; both animals were from the same locality).

The first stage in the process of degeneration consists in an increase in the size of the nucleus, with a well-marked peripheral thickening. All stages intermediate between those shown in text-fig. A, 1 and text-fig. B were to be found. During this process, there does not appear to be any considerable increase in the actual amount of chromatin present in the nucleus: for though the degenerate nucleus often attains twice

TEXT-FIG. B.



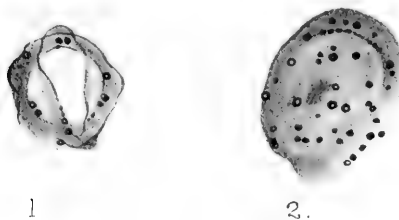
E. ranarum, an individual in process of degeneration. N = Nucleus
F = Food bodies.

the diameter of the normal nucleus, it nevertheless stains much less deeply with chromatin stains (compare text-figs. A, 1 and 2). The central part is usually almost free from chromatin in the degenerating animals (see text-fig. C, 1). During these morphological changes in the nucleus, chemical changes also take place. A number of refractive granules make their appearance in the nucleus (text-fig. A, 2, etc.). These granules do not take up the nuclear stain, and are very distinct in the living animal. In later stages of degeneration, they are replaced by granules of brown pigment: but whether they are directly converted into pigment, or whether the pigment is a

subsequent formation, I am unable to say with certainty. It appears to me probable that the refractive granules are directly transmuted into pigment. In organisms which have degenerated to a considerable extent, a great deal of pigment may be present in the nucleus (see text-fig. D, 2).

As degeneration proceeds, the nucleus becomes again modified. It tends to become a more uniformly staining mass (text-fig. C, 2), losing its foldings and distinct peripheral thickening. It seems to be undergoing a process of dissolution at this stage. From the fact that many such nuclei were found lying freely in the gut of the host (together with

TEXT-FIG. C.



Degeneration nuclei.

enucleate amœbæ), it seems probable that the nucleus may be bodily ejected from the cell at this period. However, I have not observed this in the living animal.¹

Certainly, in many cases the nucleus now undergoes absorption—the process proceeding until only the granules of brown pigment are left (text-fig. E, 1). These amœbæ are still quite active, and have a very curious appearance whilst alive.

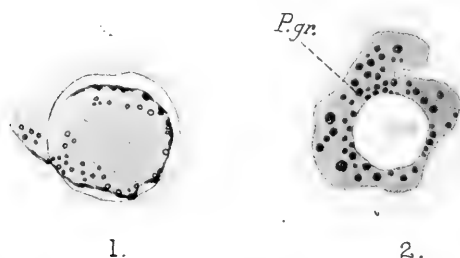
Even the pigment, however, may totally disappear, and amœbæ result which show no vestiges of the nucleus which was originally present (see text-fig. E, 2). These enucleate forms in their last stages, contain practically no food material. It is remarkable that, although in early stages of degeneration the amœbæ are often full of food bodies in various stages of

¹ Prandtl (11) describes this as occurring in *Amœba proteus*.

digestion (cf. text-fig. B), yet as degeneration proceeds no more food is ingested, and that originally present is but slowly digested. A few undigested remains of food are often seen in the enucleate forms (see text-fig. E, 2), but these are often quite hyaline and free from all trace of cytoplasmic inclusions.

How long these organisms are able to remain alive in this condition when inside their host, I am unable to say. I have observed them undergoing active, and apparently quite normal, movements for many hours under the microscope, before death finally supervened. There is no difficulty in making such observations on the living animals, for—with proper

TEXT-FIG. D.



Degeneration nuclei; in optical section. *P. gr.* = Pigment granules.

procedure—the structure can be observed in the living animal with as much precision as in a fixed and stained preparation.

There is absolutely no evidence to show that the amœbæ are capable of recovery after the processes of degeneration which I have just described have once set in. I should also point out that this kind of degeneration and death differs entirely from that which happens to an ordinary individual when removed from its host. It is also quite different from the simple degenerative changes which sometimes occur in hypertrophied animals which have been kept for some days in cultures of the fæces (see 2, p. 249).

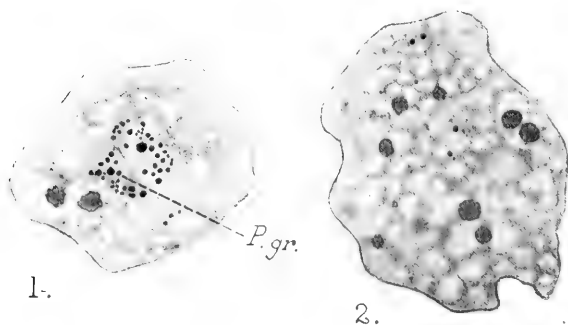
On several occasions I have found degenerate nuclei of the type seen in text-fig. D, 1. In these the whole of the central

mass of nuclear matter has shrunk away from the membrane. I am not sure what takes place subsequently, but it appears probable that such nuclei undergo a simple process of disintegration.

II.

More than thirty years ago, Brandt showed that enucleate fragments of *Actinosphærium* invariably die. And many subsequent workers—Nussbaum, Gruber, Hofer, Verworn, Balbiani, and others—have proved that this rule is of general

TEXT-FIG. E.



Enucleate amœbæ, in late stages of degeneration. *P. gr.* = Pigment granules.

application. They have proved by direct experiment that if a protozoon be divided into two parts—one containing the nucleus, the other enucleate—then only the nucleate part is capable of carrying out the vital functions of the animal for any length of time. The enucleate part invariably dies—sooner or later. Of especial interest in the present case is the work of Hofer (10). This investigator experimented upon the free-living *Amœba proteus*. He cut the organism into two parts—one of which contained the nucleus, the other being enucleate. By keeping both parts under constant observation he found that the nucleate part continued to live and perform its various functions in the normal manner. The enucleate

part, on the other hand, though it remained capable of movement for many days, invariably died in the end. Whilst it continued to live, however, it was seen to be incapable of ingesting food material, and apparently had but little power to digest further such food as was already present. Hofer concluded that the power of locomotion exists in the cytoplasm, independent of the nucleus: but that ingestion and digestion of food by the cytoplasm are possible only with the co-operation of the nucleus.

These two important conclusions are supported by the facts which I have just described in *Entamœba ranarum*. Enucleation in this case, however, is gradual, so that the change of properties in the resulting organism is not so sharply marked as in the case of a vivisected amœba. But the resulting animal, without a nucleus, is—as we have seen—capable of locomotion, but incapable of ingesting and assimilating food.

The phenomena of physiological degeneration and death which I have described in *E. ranarum* in the preceding section, are paralleled in other Protozoa (cf. Hertwig [4, 6, 8], etc., Dobell [1, 3]). The most striking parallel is seen in the case of *Amœba proteus*, which has been shown by Prandtl (11) to undergo a process of physiological degeneration very similar to that which I have observed in *E. ranarum*. I wish here to say a few words regarding the cause and significance of these phenomena. As the majority of the facts have been admirably dealt with already by R. Hertwig, I will limit myself to as few remarks as possible.

Richard Hertwig, who originated the term “physiological degeneration” for these phenomena, seeks to elucidate them by means of his hypothesis of the karyoplasmic relation (“Kernplasmarelationstheorie”). The degeneration is the result of the overgrowth of the nucleus as compared with the cytoplasm. If this overgrowth is not corrected—e. g. by the elimination of nuclear matter—then death results. Hertwig’s own observations upon *Actinosphaerium*, *Dileptus*, *Paramecium*, etc., speak strongly in favour of this interpretation.

The overgrowth of the nucleus, resulting in a condition of depression or physiological degeneration, may be induced, according to Hertwig, either by over-feeding or by starvation, and also by change of temperature. Let us consider these factors in the case of *E. ranarum*.

In the first place, it appears to me that neither excess nor deficiency of nutriment can be the cause of physiological degeneration in *Entamoeba ranarum*.¹ I usually found considerable numbers of amoebæ undergoing degeneration together, and among these it was always easy to find all forms from those literally packed with food (cf. fig. B) to those containing practically none. Although the degenerate amoebæ occurred together in large numbers, I cannot believe that an excessive metabolic activity, which accompanied the preceding period of rapid multiplication, could be the cause of degeneration. For I have frequently found perfectly normal individuals present together in equally large numbers.

With regard to temperature, the facts are interesting, though inconclusive. I find on referring to my note-book that the degenerate amoebæ which I have found occurred in January (1908, 1909) and February (1907). Now I believe the development of *E. ranarum* in the frog normally culminates in encystation. And it is only in the months of December, January, and February that I have ever found encysting animals (see 2). Before encystation, as I have already recorded (2), a very curious process takes place—an elimination of a considerable amount of chromatin from the nucleus. Now, as has already been shown by Hertwig and his school, in many Protozoa the size of the nucleus—as compared with

¹ Prandtl (11) apparently attributed the physiological degeneration which he observed in *Amœba proteus* to active multiplication, through prolongation of suitable conditions, at a time when multiplication—in the ordinary course of events—would have ceased. The *Amœbæ* were collected in autumn—"also gegen Schluss der Vermehrungsperiode der meisten freilebenden Protozoen. Indem nun die Tiere durch die gebotenen günstigen Vermehrungsbedingungen zu weiteren Theilungen veranlasst wurden, gingen sie ihrer physiologischen Degeneration entgegen."

the cytoplasm—increases with lowering of temperature.¹ This at once suggests that the events preceding encystation in *E. ranarum* are somewhat as follows: In winter the lowering of the temperature leads to an acceleration in the growth of the nucleus. In the ordinary course of events the nucleus then regulates its size by extruding a quantity of nuclear matter into the cytoplasm. This act then calls forth in the organism those changes which bring about encystation.

The occurrence of physiological degeneration could thus be explained as follows: When the lowering of temperature occurs, some unknown factor acting upon the amœba prevents the elimination of the excess of nuclear material produced. This, therefore, would lead to considerable increase in the size of the nucleus—which, as I have shown, actually occurs. As the nuclear material is not given up to the cytoplasm the factor which determines encystation does not come into play, so that the animal does not encyst. The excess of nuclear material is gradually turned into pigment, etc., and the unencysted animal finds itself in a highly abnormal condition, from which it is unable to recover. It therefore undergoes degeneration and death.

This gives us, I think, a plausible explanation of the phenomena which I have observed. There yet remains, however, the question—at present unanswerable—What is the factor which, *ex hypothesi*, prevents the regulation, by elimination of nuclear matter, of the size of the nucleus, and in consequence, prevents encystation and causes death? It is conceivably some toxic body—e. g. a secretion of the host, or the metabolites of the amœbæ themselves, or a toxin produced by the large numbers of bacteria in the host's gut. On the other hand, it may be a factor which lies within the amœba²

¹ See, for example, Popoff, "Experimentelle Zellstudien," 'Arch. f. Zellforschung,' Bd. 1, 1908. Lowering of temperature caused both an absolute and a relative increase in the size of the nucleus in the Infusoria studied.

² That the overgrowth of the nucleus is itself the primary cause of degeneration and death appears to me highly improbable. I would as soon argue that grey hairs are the cause of old age in man.

—in other words, the organism may lose its power of vital regulation, and so die “naturally.”

This last is at least possible. With increase of knowledge it has become necessary to modify considerably the old conception—first precisely stated by Weismann—that the Protista are immortal. We now know that cell-death—complete or partial—is a common phenomenon in the Protozoa. Many of the facts have been already expressed far better than I could express them by Richard Hertwig (see 5, 9, etc.). And he concludes: “Im Gegensatz zu Weismann nehme ich an, dass schon im normalen Lebensprozess die Keime des Todes enthalten sind, dass der Tod keine zufällige Anpassung ist, sondern die nothwendige Consequenz des Lebens selbst. Somit können auch die Protozoen nicht unsterblich sein in dem Sinne wie Weismann will; sie würden ebenso zu Grunde gehen müssen wie die vielzelligen Thiere, wenn nicht Einrichtungen getroffen wären, welche die schädlichen Wirkungen des Lebensprocesses compensiren” (5, p. 73).

[All the figures are drawn from permanent preparations fixed with sublimate alcohol, and stained with Delafield's hæmatoxylin and eosin. They were all drawn under the Zeiss 2 mm. apochromatic oil imm. (1.40) with compens. oc. 12.]

ZOOLOGICAL LABORATORY,
CAMBRIDGE,
April, 1909.

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11. Prandtl, H.—“Die Physiologische Degeneration der *Amœba proteus*,” ‘Arch. Protistenk.,’ viii, 1907, p. 281.

Observations on the *Amœbæ* in the Intestines of Persons Suffering from Goitre in Gilgit.

By

Robert McCarrison, M.D., M.R.C.P.Lond.,
Captain Indian Medical Service.

With 24 Text-figures.

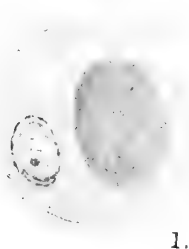
My researches on the ætiology of endemic goitre have led me to the conclusion that in all probability the organism which is responsible for the production of this disease is to be found in the intestinal tract. It is necessary, therefore, to give some account of the Protozoa which are so frequently present in this situation. This paper deals only with the organisms of the *Amœba* group, and simply gives a brief description and figures of the amœbæ found. It does not claim to be a complete account of their life-histories. While no definite statement can be made as to the pathogenicity of these amœbæ, their possible importance is obvious when it is remembered that goitre is due to an organism carried by water.

The examination of the fresh fæces of goitrous individuals was undertaken solely for the purpose of determining the presence or absence of Protozoa. This object was found to be greatly facilitated by the addition of a small quantity of iodine-water to the specimen, which caused the amœbæ to stand out clearly from surrounding objects. One hundred and three cases were examined in this way; amœbæ were present in eighty-seven. In forty-eight cases they were found in large numbers, in twenty-seven in moderate numbers, and in twelve only after considerable time had been spent in

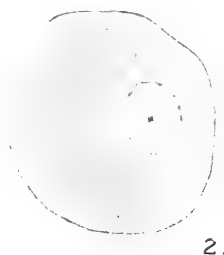
searching for them. The fæces of 101 non-goitrous individuals, living in the same locality, have also been examined. Amœbæ were present in twenty-nine of these; the infection was plentiful in eight, moderate in nine, and scanty in twelve. The typical cysts hereafter described were also found in the only case of goitre from another locality which I have examined.

Two distinct amœbæ are found in the fæces; these have not been distinguished in the live state:

(1) A free amœba which proceeds to encyst and develop into a typical 8-nucleated cyst.

TEXT-FIG. 1.¹

TEXT-FIG. 2.



Text-fig. 1.—Amœba I. Encysted amœba, showing single nucleus and port-wine staining area.

Text-fig. 2.—Amœba I. Encysted amœba. Shows the kidney shape of the port-wine staining area frequently observed.

(2) A free amœba which does not form obvious cysts, but multiplies by division and budding.

In addition, a third amœboid body, enclosed in a characteristic capsule, is also present. Its affinities are not clear, and I can only note its occurrence.

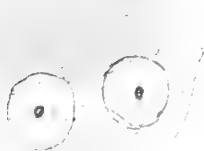
¹ [Figs. 1-7 and 23 are drawn from freehand sketches of the living animals. The preparations were treated with iodine water. Drawings made under Leitz $\frac{1}{12}$ in. oil-immersion, ocular No. 4.

The remaining figures were drawn from fixed and stained preparations, under Zeiss 3 mm. apochromatic homog. oil-immersion, comp. oc. 12 ($\times 2000$). The drawings were made by Miss Rhodes, to whose skill in so accurately depicting the appearances observed I must pay a tribute. The preparations were stained with Haidenhain's hæmatoxylin and Delafield's hæmatoxylin.]

THE AMŒBÆ IN THE FRESH STATE.

(a) The free amœbæ.—I can only give a general account of the free amœbæ in the live state, an account which covers both species. In specimens treated with iodine solution the organisms are more or less spherical. Their size varies between $12\ \mu$ and $20\ \mu$. Larger forms are occasionally seen. The protoplasm is granular, stains yellow with iodine, and is very rarely vacuolated. A differentiation of the protoplasm into ectoplasm and endoplasm can only be made out in those animals which show pseudopodia, and these are few. The

TEXT-FIG. 3.



3.

TEXT-FIG. 4.



4.

Text-fig. 3.—Amœba I. Encysted amœba. Bi-nucleated stage with large port-wine staining area.

Text-fig. 4.—Amœba I. Encysted amœba. A stage not commonly met with, showing four nuclei and centrally placed port-wine staining area.

protoplasm contains food-vacuoles and other inclusions. I have never seen any evidence of the ingestion of blood-corpuscles or of epithelium. The nucleus, where observed, is spherical or oval, and is sometimes surrounded by a narrow halo. In the larger organisms it measured $5\ \mu$ – $8\ \mu$. The characters of the nucleus are very distinct in the two species; they have been studied only in stained specimens.

(b) The encysted forms.—Live cysts of definite contour are very commonly seen; they represent stages in the life-history of Amœba I, and show different appearances dependent on the length of time the animal has been encysted. These cysts are, as a rule, perfectly spherical, but they may be oval.

Their size varies from $14\ \mu$ – $20\ \mu$, the latter being the almost constant diameter of the 8-nucleated forms. The cyst-wall varies in thickness, and sometimes it is made out with difficulty: It encloses a yellow staining granular protoplasm, one or more nuclei, and a characteristic port-wine staining area when treated with iodine (text-figs. 1 to 4). The nucleus is spherical, very clearly defined, and shows refractile granules on its surface and in its interior. A well-marked karyosome is usually present. The nucleus varies in size from $2\ \mu$ – $3\ \mu$ in the 8-nucleated cysts to $6\ \mu$ – $8\ \mu$ in the single-nucleated

TEXT-FIG. 5.



5.

TEXT-FIG. 6.



6.

Text-fig. 5.—*Amœba* I. Encysted *amœba*. The nuclear division has resulted in the formation of five nuclei; compare text-fig. 17.

Text-fig. 6.—*Amœba* I. Encysted *amœba*, 8-nucleated stage, cyst-wall ill-defined; a centrally placed remnant of the port-wine staining area is seen—a very uncommon appearance at this stage.

form. The nuclei vary from one to eight in number, dependent on the phase of development of the cyst (text-figs. 1 to 7). The commonest phases met with are those where the cyst contains one, two, or eight nuclei. Cysts showing four nuclei are much less commonly found. The port-wine staining area is present in the majority of all cysts containing one to four nuclei (text-figs. 1 to 4). It is not, as a rule, present in the 8-nucleated cyst; I have only met with it at this stage of development in one instance (text-fig. 6). It is oval, spherical, or kidney-shaped in form, and occupies about one half of the

cyst. It usually lies in one hemisphere while the protoplasm is situated in the other, but it may be centrally placed. It appears sometimes to alter its position relative to the nuclei and to the cyst-wall. The port-wine reaction with iodine marks off the area from the rest of the protoplasm in a most distinctive way, for while the protoplasm is granular and stains yellow this area appears to be structureless.

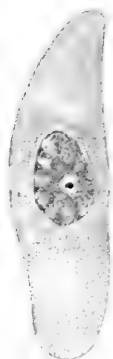
I have never been able to observe a division of the protoplasm around the nuclei in the large 8-nucleated cysts.

TEXT-FIG. 8.

TEXT-FIG. 7.

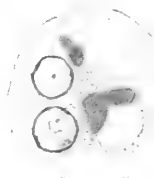


7.



8.

TEXT-FIG. 9.



9.

Text-fig. 7.—Amœba I. Encysted amœba. The nuclear division has resulted in the formation of five nuclei; compare text-fig. 17.

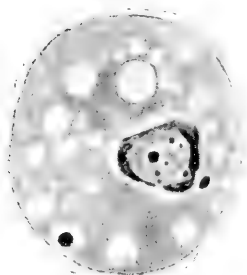
Text-fig. 8.—Amœba I. Free amœba. Sideview of organism showing finely granular appearance of protoplasm. The nucleus is seen surrounded by a narrow halo, and shows chromatin more or less evenly distributed with slight massing at four points of the periphery. A central karyosome is seen.

Text-fig. 9.—Amœba I. Unencysted amœba. Probably a stage in simple fission of free amœba. Chromatin massed at periphery of nuclei, which have beaded appearance. Karyosome is seen; nature of included bodies not known.

(c) The third organism referred to is less commonly found. The following are its main characteristics in both fresh and stained specimens. In the living state it is seen as a pear-shaped, oval, or spherical body, having a well-defined clear capsule. The protoplasm is granular and stains yellow with iodine. It is split up by a median fissure, on either side of

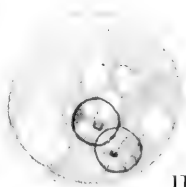
which and approximately at right angles to it is a shorter fissure, which further divides up the protoplasm (text-fig. 23). Movements of the protoplasm and alterations in the position of the nuclei within the capsule are sometimes seen. The nuclei, four in number, usually lie clumped together or in the position shown in the figure (text-fig. 23). They are clear, spherical bodies of uniform size, very sharply defined, and sometimes showing a central dot, features which are well brought out by staining (text-fig. 24). When the organism is

TEXT-FIG. 10.



10.

TEXT-FIG. 11.



11.

TEXT-FIG. 12.



12.

Text-fig. 10.—*Amœba* I. Encysted *amœba*. Protoplasm filled with spherical hyaline masses of variable size. Two chromatin masses are seen in the protoplasm. Chromatin heaped up at opposite poles of nucleus; karyosome well marked.

Text-fig. 11.—*Amœba* I. Encysted *amœba*. Protoplasm shows hyaline masses of larger size. Nucleus has divided into two. Reticular structure of nucleus and karyosome seen.

Text-fig. 12.—*Amœba* I. Encysted *amœba*. Protoplasm shows single hyaline mass. Nuclei as in Text-fig. 11, but of larger size.

stained the capsule is not coloured, and it appears to be open at its broader end. The granular protoplasm sometimes stains so deeply that no structure can be made out. In the more faintly stained animals the protoplasmic fissures referred to can be seen, but not with such distinctness as in the living animal (text-fig. 24). Sometimes the protoplasm, with its contents, is seen contracted up at one side of the capsule. The size of this animal in its longest diameter is fairly constant, and measures in the live state 14μ – 15μ .

THE FIXED AND STAINED AMEBÆ.

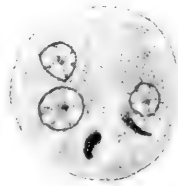
The material used for this part of the investigation was sent from Gilgit to England in Schaudinn's fixing reagent (saturated watery solution of corrosive sublimate two parts, alcohol one part). The methods of staining employed were Delafield's and Haidenhain's hæmatoxylin. The examination of the material was carried out in Professor Minchin's laboratory at the Lister Institute, to whom I am indebted for help and advice.

TEXT-FIG. 13.



13.

TEXT-FIG. 14.



14.

Text-fig. 13.—Amœba I. Encysted amœba. Shows well-marked cyst-wall. A large hyaline mass occupying about one half of the cyst is seen. Chromatin masses lie above and below this area. Nuclei are seen lying at either side of cyst, each surrounded by halo. Note the reticular structure and granular appearance of nuclei. No karyosome is seen in either nucleus.

Text-fig. 14.—Amœba I. Encysted amœba. One nucleus has proceeded to second division before the other. A common appearance of the nuclei—wheel-like—at this stage is well seen. Two chromatin masses in protoplasm.

A study of the stained amœbæ shows that there are two distinct species present. In view of the many opinions held with regard to intestinal amœbæ I hesitate to describe these organisms under specific names. Nevertheless, I am inclined to think that Amœba I, which forms the 8-nucleated cysts, is the *Entamœba coli*, Schaudinn, and that Amœba II corresponds to the *Entamœba histolytica*, Schaudinn. Certain points in which they appear to differ from the two

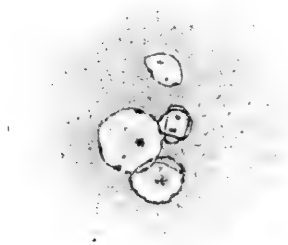
species of Schaudinn will be referred to in the course of my description.

Amœba I.

The unencysted amœba usually appears as a spherical body of variable size, ranging up to 20μ in diameter. The protoplasm is finely and evenly granular (text-fig. 8). I can detect no differentiation into ectoplasm and endoplasm.

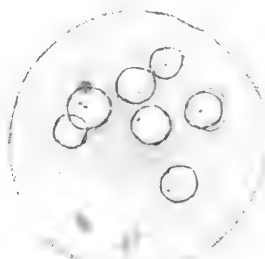
In some the protoplasm contains food-material and various inclusions, while in others it appears to be free from

TEXT-FIG. 15.



15.

TEXT-FIG. 16.



16.

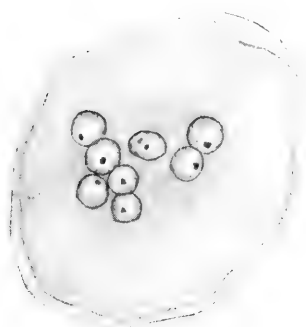
Text-fig. 15.—Amœba I. Encysted amœba. The nuclear division has resulted in the formation of five nuclei.

Text-fig. 16.—Amœba I. Encysted amœba. Typical 8-nucleated cyst, of very common occurrence in faeces. Each nucleus is ring-like with a small karyosome in its interior. A few small chromatin masses are seen.

extraneous matter. Such inclusions as blood-corpuscles or epithelium have never been met with. The protoplasm rarely shows a vacuole. The nucleus is very distinct, and is usually centrally placed (text-fig. 8). It is commonly surrounded by a narrow but distinct halo. In text-fig. 8 the nucleus appears to lie in a cavity lined by a membrane. Since it has been preserved in a sublimate mixture the appearance may be due to shrinkage of the protoplasm. The nucleus stains deeply; it is rich in chromatin, which in the adult unencysted animal is more or less uniformly distributed throughout this structure. There is often a slight tendency for the chromatin to be

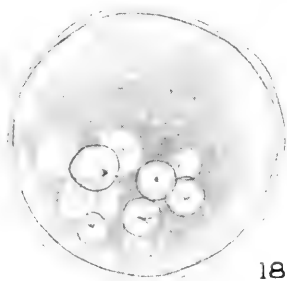
massed irregularly at the periphery of the nucleus. It is generally reticulate in character, and, as a rule, shows a distinct karyosome. I have not been able to satisfy myself as to whether division of the nucleus takes place in the unencysted organism or not. Text-fig. 9 represents an organism of a type only occasionally met with, in which it was impossible to make out the existence of a cyst-wall. This is probably a stage in simple fission of the free amœba. Nevertheless I hesitate to offer an opinion on the point, and simply draw attention to the figure. One point is certain, that in all

TEXT-FIG. 17.



17.

TEXT-FIG. 18.



18.

Text-fig. 17.—Amœba I. Encysted amœba; 8 nucleated cyst.

The cyst-wall is thicker than in text-fig. 16. The nuclei are very clearly defined and each shows a karyosome.

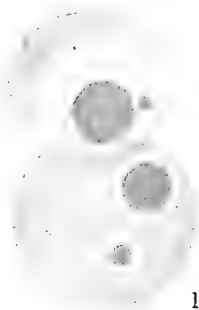
Text-fig. 18.—Amœba I. Encysted amœba. Abnormal form showing twelve nuclei.

organisms in which there were more than two nuclei a cyst-wall was always obvious. Unfortunately I have not noted a similar appearance in the living state, but, as I have said, my observations were then only diagnostic.

The encysted amœba.—The earliest stages of encystment which I have observed are shown in text-fig. 10. The chromatin is heaped up at the periphery of the nucleus, and here and there in the protoplasm dark masses presenting staining reactions similar to those of chromatin are occasionally found. The appearance of the protoplasm is dis-

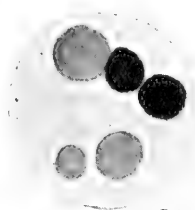
tinctive; it seems to be filled with spherical hyaline masses of variable size. It is difficult to offer an opinion as to the nature of these spherical masses. Such text-figures as 11 and 12 suggest that they gradually fuse, with the ultimate formation of a large, clear hyaline body, as seen in text-fig. 13. This fusion appears to take place in those stages which represent the division of the nucleus after encystation. I am convinced that the clear area (text-fig. 13) is that which gives rise to the characteristic port-wine reaction with iodine water in the living animal. The hyaline body becomes less marked as

TEXT-FIG. 19.



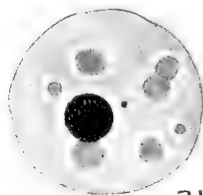
19.

TEXT-FIG. 20.



20.

TEXT-FIG. 21.



21.

Text-fig. 19.—*Amœba* II. Two typical organisms. Shows pale staining nuclei surrounded by narrow halo.

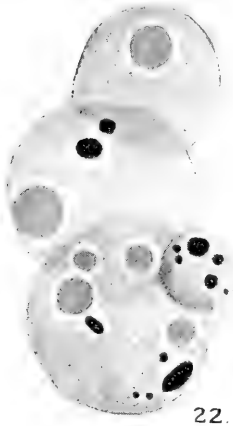
Text-fig. 20.—*Amœba* II. Organism showing three nuclei and two darkly staining bodies. The precise nature of the latter objects is unknown; probably food-material.

Text-fig. 21.—*Amœba* II. A multi-nucleated organism. Nuclei of varying sizes. A stage of multiple division.

the further development of the organism proceeds to the typical 8-nucleated form; ultimately it completely disappears. This hyaline body is at its highest development in the late bi-nucleated cyst. I regard this spherical mass as being of the nature of food material, a view which is upheld by Jurgens (2). A similar appearance has been described by Wenyon (3) in *Entamoeba muris*, and he has considered it to be "of the nature of food products which have not been thrown out of the animal." It is questionable, however, whether the body seen by Wenyon is identical with that

which I am describing. Wenyon has found that the refractile body of *E. muris* "stains feebly, and shows a coarse reticular structure," and that on breaking up "the separate parts shrink to form masses which stain deeply with hæmatoxylin." The hyaline body I have described has not these characters. Schaudinn (4) has described the protoplasm of the encysted *E. coli* as "divisible into an outer and denser layer containing the nucleus and an inner and more liquid portion,"

TEXT-FIG. 22.



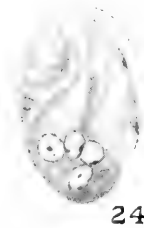
22.

TEXT-FIG. 23.



23.

TEXT-FIG. 24.



24.

Text-fig. 22.—Amœba II. Group of amœbæ, the result of multiple division; a common appearance.

Text-fig. 23.—The third amœboid body. Shows the characteristic capsule and fissured protoplasm as observed in the fresh state. The drawing also shows a common position of the four spherical nuclei.

Text-fig. 24.—The third amœboid body. Shows the typical appearance of this organism when stained. Note the slightly beaded appearance of the nuclei, the presence of karyosomes, the position of the nuclei, and the manner in which they lie clumped together. The unstained capsule and the fissured protoplasm is well seen.

and Wenyon considers that the more liquid portion probably corresponds to the refractile body of *E. muris*. It does not, however, correspond to the hyaline body of the amœba under consideration. The characteristic port-wine reaction differentiates it from the yellow-staining granular protoplasm. This hyaline body is a conspicuous feature of the great majority

of encysted amœbæ during the earlier phases of their development.

At the time of encystment there is one nucleus present (text-fig. 10). This nucleus divides into two daughter-nuclei (text-figs. 11, 12). I have not been able to trace the complicated series of nuclear changes described by Schaudinn in *E. coli* and by Wenyon in *E. muris* as occurring at this stage, though I have seen a large number of amœbæ. The two daughter-nuclei lie most commonly side by side (text-figs. 11, 12). At this stage they vary greatly in size. I have seen them so large their diameter almost equalled half that of the cyst containing them. I have observed in one case a division-figure corresponding closely to fig. 73 of Dobell's paper (5); unfortunately the specimen could not be drawn. In text-fig. 13 the two nuclei have separated to either side of the cyst. Division of the two daughter-nuclei takes place, and text-fig. 14 shows that one nucleus has proceeded to the second division before the other. It has been difficult to find examples showing four nuclei; text-fig. 15 shows a further division of the nuclei into five. The ultimate division into eight is shown in text-fig. 16. The cyst-wall eventually becomes thickened as in text-fig. 17. Division of the protoplasm around the nuclei has never been seen. Very rarely a form showing more than eight nuclei is met with (text-fig. 18), but it is so rare that we must regard it as abnormal.

It is evident that the animal here described corresponds very closely to the *Entamœba coli* of Schaudinn and to the *Entamœba muris* of Wenyon. The descriptions, however, do not correspond as regards the hyaline body, which is so characteristic of this organism; nor have I been able to trace in it the nuclear changes described by these observers.

Amœba II.

Amœbæ of this species are exceedingly plentiful in some cases; as many as three or four are often found in one field of the microscope. They may occur alone or in association

with one or other of those described in the preceding sections.

The animal is usually spherical and of variable size, the forms most commonly met with averaging 15μ – 20μ in diameter. The protoplasm is sometimes divisible into a granular endoplasm with a thin layer of ectoplasm surrounding it. The ectoplasm is most obvious in these rare cases in which protrusions in the form of pseudopodia are seen. Often it is not very clearly marked off. The endoplasm is finely granular and contains extraneous matter; such inclusions as blood-corpuscles or epithelium have never been seen. I have not observed vacuoles. The nucleus in some cases is very difficult to see, presenting as it does staining reactions differing only a little from those of the protoplasm. It is frequently surrounded by a clear halo (text-fig. 19). It is very poor in chromatin and does not appear to possess a karyosome. There is nothing distinctive in the position of the nucleus; it may be central, but is more frequently excentric. It varies very considerably in size. Forms are commonly seen containing two or more nuclei (text-figs. 20, 21). Multiplication is apparently very rapid, and takes place either by a process of simple division or by multiple division of the nuclei with budding of the protoplasm, examples of which processes are shown in text-figs. 19 and 22). This organism does not encyst as does *Amœba* I. I have been unable to find that it develops cysts such as Schaudinn has described in *E. histolytica*. It is true that I have frequently found small, dark brown, spherical bodies in preparations where this was the only amœba present; their structure could not be made out, nor could it be shown that they had any connection with this amœba.

This organism resembles closely the *E. histolytica* of Schaudinn. There was, however, no evidence that patients whose fæces swarmed with this *Amœba* were suffering from dysentery.

I would also like to point out that the amœba here des-

cribed is much smaller in size than the *Entamoeba histolytica* of Schaudinn.

LISTER INSTITUTE,

March 9th, 1909.

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Peripatus ceramensis, n. sp.

By

F. Muir and J. C. Kershaw.

With Plate 19.

Female.—Antennæ dark grey, the articulations thinly ringed with ochreous. Eyes shiny black. Oral papillæ pale ochreous at the base, pale grey on tips, and dotted there with black. Head and anterior part of body to about the second pair of legs rather pale rust-colour, irrorated with black; there is in some specimens an ochreous marking on the dorsal median line of the head. Rest of body dark slaty-grey, irregularly spotted with small sub-circular rust-coloured spots, most of them centred with a black dot (the spots are the bases of papillæ, the black dots the bristles at the summits). These spots extend to near the head, but are there smaller and paler. There is a narrow median dorsal line of very dark grey, becoming narrower, paler, and almost obsolete near the head, but it continues fairly distinct to the anus. This line, under the microscope, is seen to be divided longitudinally by an exceedingly fine light line. Underside wholly grey, lighter than the upperside, and irregularly spotted with pale ochreous, chiefly on each side of the median line. Round the mouth is pale ochreous. Legs on the inside concolourous with the underside; on the outside concolourous with the upperside, and with a few pale rusty spots, chiefly near the base. Just around the anus the skin and papillæ are ochreous or rusty. The spiniferous pads on the legs are pale ochreous. Between each pair of legs, on

the median ventral line, is a rather large light ochreous spot. Papillæ round genital opening whitish.

The newly-born young are uniformly pale dove-grey, and take four or five days to become dark, and several days longer before the spots appear; at first being pale ochreous, and not becoming rust-colour till much later in life.

Out of sixty-three specimens examined one had twenty-two pair of legs, the remainder only twenty-one; they decrease in size towards the ends, especially posteriorly, where the last pair are very small (fig. 3). Three spiniferous pads on each leg, except the last pair, where they are missing or only rudimentary; the basal pads of the fourth and fifth pairs of legs divided into three parts; the opening of the modified nephridia situated on the central portion. Foot with three primary papillæ, one dorsal and two lateral, one on each side (fig. 2); on the last pair the dorsal papillæ are nearer to the anterior lateral ones.

When proceeding over plane surfaces the animal walks on the spiniferous pads, the feet being turned back on the dorsal side of the leg, and only brought into play when it is necessary to use the claws as hooks. The last pair of legs is not used for walking, and very often the penultimate pair do not come into play.

Inner jaw-claw with six accessory teeth, outer jaw-claw with none. "Tongue" with six small teeth, the anterior two side by side, the four behind in a longitudinal row.

Vagina, or genital opening, subterminal, behind the last pair of legs, and near to the anus (fig. 3).

The female genital organs consist of two long tubes, which meet together posteriorly to form the very short duct leading to the vagina, and anteriorly where they form the ovaries (fig. 4).

The ovaries consist of a bilobate chamber about 1 mm. long, with morula-like walls filled with nucleated cells of various sizes which causes it to vary slightly in size and shape. It is attached to the median line of the septum, between the seventeenth and eighteenth pair of legs, by a

thin membrane (fig. 5, *m.*); a pair of fine tracheæ run along the edges of this membrane and enter the ovaries. A single short median duct leads from the ovarian chamber and immediately divides into two oviducts (fig. 5, *ovd.*). At the end of each oviduct is a small globular receptaculum seminis opening into the oviduct by two short ducts (fig. 5, *r.s.* and *d.*); beyond these the tubes increase in size to form the large uteri (fig. 4, *ut.*).

In very young specimens these organs are coiled up in the central body cavity behind the seventeenth or eighteenth pair of legs, the ovaries are attached by a short membrane to the septum, and the tubes are of equal diameter throughout. As they develop the membrane lengthens, and the ovaries and receptacula seminis are carried forward to about the fourteenth or fifteenth pair of legs, the uteri continue on till about the tenth or eleventh and then bend back and proceed to the subterminal vagina, increasing in size from the receptaculum seminis backwards.

The eggs are very minute, about .05 mm. in diameter; after passing the receptaculum seminis they begin to swell and divide, and embryos in various stages of development are found in the uteri, the one nearest the vagina in one uterus being slightly more developed than the corresponding one in the other uterus. The young are born one at a time, apparently alternately from each uterus, at intervals of about a couple of weeks; most likely births take place the whole year round. The largest specimen we took was about 55 mm.; the young when born are about 13 or 14 mm.

The sixty-three specimens examined were all females, so the male remains still to be discovered. That the male of such a lethargic animal should be so rare is very strange. Many of the small individuals had embryos in their uteri showing that they had been fertilised; three half-grown specimens had their genital organs still small and packed away behind the seventeenth pair of legs and evidently had not been fertilised; in these three specimens the slime ducts were enormously distended.

All these specimens were taken in the vicinity of Përoe (Western Ceram) living in old logs and stumps of trees in a certain stage of decay; in one case just under the bark, but in all the others some distance below the surface, working their way in along cracks and runs made by insects. In captivity their favourite food is the pupæ of wood-boring beetles, which they bite open and suck out the contents.

This species, the first taken in the Moluccas, appears by the female characters to differ from all others and to form a distinct group. In the size of the eggs and its mode of development and birth it approaches the neotropical group but is quite distinct in all other characters, such as number of legs, position of vagina, shape of papillæ and number of pads on the legs. In these latter characters it comes nearer to the South African and Australian species, but the bilobate ovarian chamber with the single duct leading from it places it quite apart. It will be of interest to see if Papuan species, when found, will agree with it.

PËROE, WEST CERAM;
March, 1909.

EXPLANATION OF PLATE 19,

Illustrating Messrs. F. Muir's and J. C. Kershaw's paper on
"Peripatus Ceramensis, n. sp."

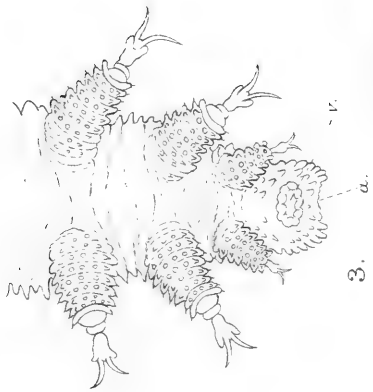
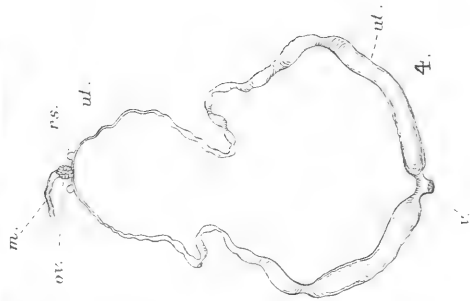
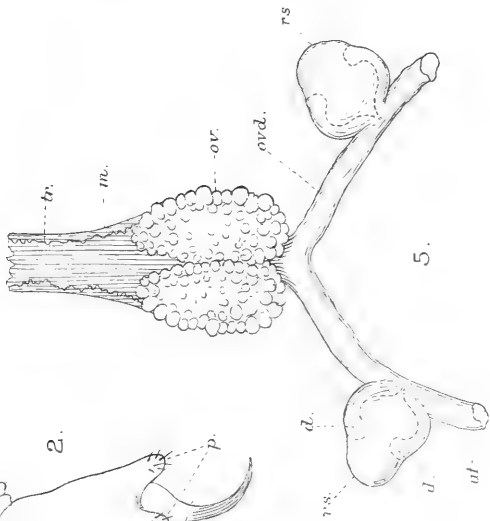
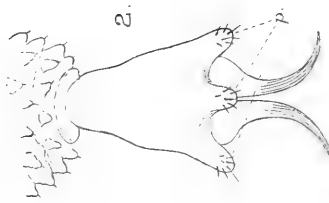
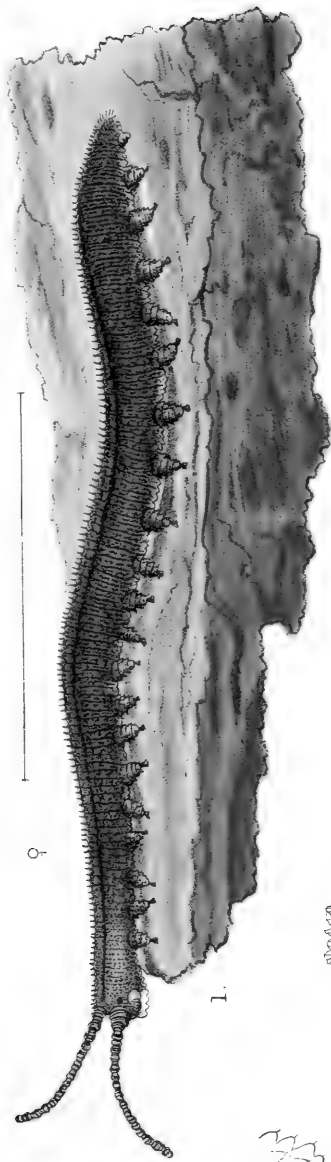
FIG. 1.—Adult female, enlarged about $2\frac{1}{2}$ times.

FIG. 2.—Dorsal view of foot. *p*. Primary papillæ.

FIG. 3.—Ventral view of last three pair of legs. *v*. Vagina or genital opening. *a*. Anus.

FIG. 4.—Female genital organs, slightly enlarged. *m*. Membrane. *ov*. Ovaries. *rs*. Receptaculum seminis. *ut*. Uterus. *v*. Vagina.

FIG. 5.—Much enlarged view of anterior end of fig. 4, with same lettering, except—*tr*. Trachea. *ovd*. Oviduct. *d*. Ducts of receptaculum seminis.



On the Eggs and Instars of Scutigereila sp.*

By

F. Muir and J. C. Kershaw.

With Text-figures.

THIS species is common in Amboina, very abundant in Ceram, and probably also in all the Moluccas, and perhaps other islands of Netherlands India. It lives chiefly, and in great numbers, in the black mould between the bark and the wood of rotten logs, in the rotten wood itself, under dead leaves, and under stones and pieces of wood lying on the ground. It is found only in damp situations, and seems to prefer the low-lying land to the hills, though it is common there also.

The female makes use of small cavities in the wood to lay her eggs in, the cavities being probably made by other wood-boring insects. The eggs (fig. 1) are laid in batches of about half a dozen; there is a short, stout pedicel or pillar, hollow and more or less ribbed, the upper part of which embraces the lower half of the first egg laid; the base of the pillar is cemented to the wall of the egg-chamber. The rest of the eggs are cemented to the first egg and to one another, and

* The authors having requested me to send the Myriapods which are the subject of this paper, to some competent naturalist, I consulted Mr. Pocock, who informs me that they belong to the genus *Scutigereila*, and probably the species *Orientalis*, Hansen (this JOURNAL, vol. 47, 1903, p. 38).—ADAM SEDGWICK.

apparently the pillar serves to keep the eggs clear of the cavity walls and allows the female to reach and examine the

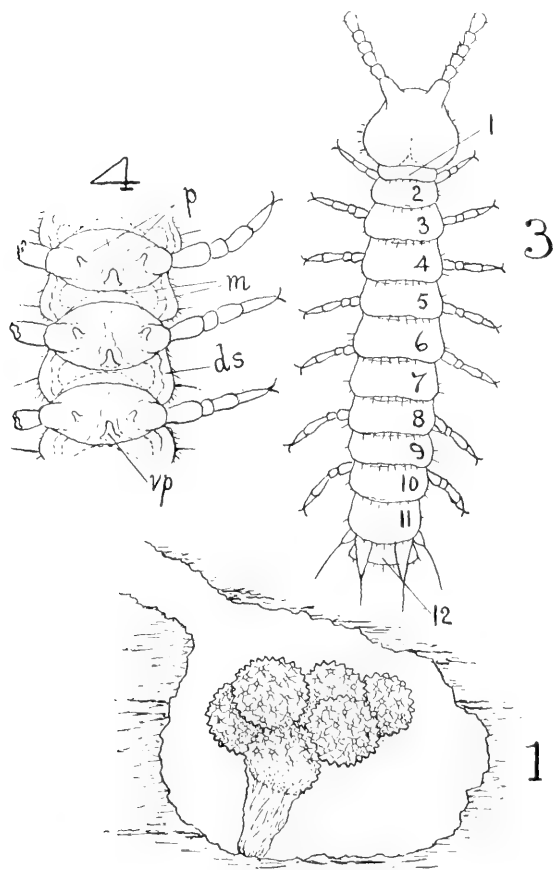


FIG. 1.—Eggs in egg-chamber.

FIG. 3.—Dorsal view of newly-hatched animal. 1—12 the tergites of the trunk segments.

FIG. 4.—Ventral view of mid-segments of adult. *p*. Semi-chitinous pedigerous sternite. *m*. Membrane connecting the sternites. *vp*. Median ventral process found on segments 4—9 inclusive. *ds*. Dorsal scutæ (tergites).

eggs—perhaps to keep them free from mould and mites. The eggs are dull white and processed all over, each process

emitting little ridges or ribs from the base (usually five ribs), which form a sort of reticulation over the surface. They are nearly globular, about $\frac{1}{2}$ mm. in diameter, and seem large in

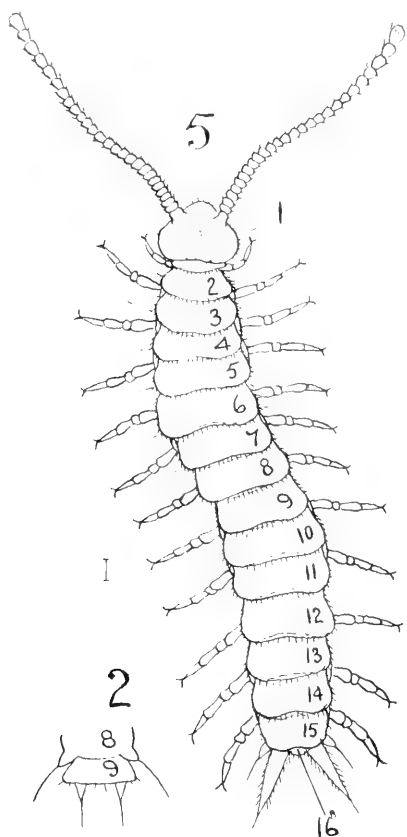


FIG. 2.—Ventral view of posterior segments (8, 9) of newly-hatched animal.

FIG. 5.—Adult female. 1—16 tergites.

comparison with the female. We took the eggs in February and March, but there are probably broods throughout the year.

The newly-hatched animals have seven pairs of legs, and acquire a pair of legs at a time till mature, when they possess

twelve pairs. In all instars this *Scolopendrella* is entirely white, and is very active—though, for a few hours after hatching, the young seem to rest on the empty egg-shells, along with their mother. When newly-hatched they are about 1 mm. in length. The female guards her eggs and young as do the Centipedes, and even when the egg-chamber is rudely broken open will not, as a rule, desert them. The adults, from an examination of the contents of the stomach, appear to feed chiefly on rotten wood and fungus growths, but probably, along with this material, swallow the minute animals abounding in the logs.

In the adult there are sixteen dorsal segments; the first segment very small, the fifteenth bearing the cerci. There are fourteen ventral segments, the thirteenth bearing the anal papillæ with tactile bristles. In the adult the sixteenth tergite and the fourteenth sternite are very obscure, being retracted into the penultimate segment; but in the newly-hatched animal (fig. 2, 9, and fig. 3, 12) they are perfectly distinct, and bear small hairs like all the anterior segments. Between the sternites there is a fold of softer membrane (fig. 4, *m.*) which allows free movements to the segments; this membrane cannot be considered as a segment. In a second and less common species of *Scolopendrella* in Ceram, the tergites are small and have a fold of membrane between them, as well as the sternites. On the ventral segments, four to nine inclusive, there are small median processes, one on each segment (*vp*, fig. 4).

From the following table it will be observed that there are six pedigerous instars, and that the young acquire the legs in pairs; the ventral segments increase in number in the same ratio as the pairs of legs, whilst the number of dorsal segments remains the same in the fifth and sixth (adult) instars. The number of antennal joints in the first instar is six, and in the second instar usually twelve, but afterwards we found the number of joints acquired in the remaining instars to vary very much. They also vary in the adults, but the usual numbers seem to be twenty-five to thirty. The

antennæ seem very subject to mutilation, and in many specimens the two antennæ did not possess an equal number of joints.

				Tergites.	Sternites.
Seven pairs of legs	.	.		12	9
Eight „	„	„	.	13	10
Nine „	„	„	.	14	11
Ten „	„	„	.	15	12
Eleven „	„	„	.	16	13
Twelve „	„	„	.	16	14

CERAM:

March 8th, 1909.

The Development of the Parasite of Oriental Sore in Cultures.

By

R. Row, M.D.Lond., D.Sc.Lond.

With Plate 20.

[THE following memoir is made up from two communications, dated respectively December, 1908, and January, 1909, which I received from Dr. Row. Each letter was accompanied by sketches and stained preparations; from the latter the figures on Pl. 20 have been drawn by my assistant, Miss Rhodes, at the Lister Institute. The account given here is, for the most part, in Dr. Row's own words; any remarks of mine are in square brackets.]

The parasites causing oriental sore were first described accurately by Wright ('Journ. Med. Research,' vol. x, 1903, p. 472), and named by him *Helcosoma tropicum*. On account of their obvious affinity (which the present memoir confirms) with the Leishman-Donovan bodies of kala-azar, Wright's bodies have been referred by subsequent writers (compare Lühe in Mense's 'Handbuch der Tropenkrankheiten,' iii, 2, 1906, p. 203) to the previously established genus *Leishmania* Ross ('Brit. Med. Journ.,' 1903, vol. i, pp. 1261 and 1401), and they now stand as *Leishmania tropica* (Wright), the only other known species of the genus being *L. donovani* (Lav. et. Mesn.), the parasite of kala-azar.

It was first discovered by Rogers ('Quart. Journ. Micr. Sci.,' vol. 48, 1904, p. 367), and confirmed by many subsequent observers, that *L. donovani* gives rise in cultures to a flagellate *Herpetomonas*-like form. So far as I am aware

no similar observations have been made for *L. tropica*, and the present memoir is the first account given of the cultural development of Wright's bodies. As will be seen, the result is very similar to that obtained for the Leishman-Donovan body. It is of some interest, however, that the method of cultivation required appears to be quite different in the two cases, a fact which indicates that the transmission and mode of development are different in the two parasites.

E. A. MINCHIN,
Rovigno, March, 1909.]

(1) Method of cultivation.—Material from the sore was planted in sterile sodium citrate solution (2 per cent.) and in blood-serum (human). Some of the cultures were left at the laboratory temperature (25° – 28° C.), and some were incubated at 35° C.

Those incubated at 35° C. and those planted in the sodium citrate solution did not show any growth; on the contrary, after twenty-four, forty-eight, and seventy-two hours they seemed to have disintegrated, so that not even a trace of the original parasites could be seen. In one case (a sodium citrate culture at room-temperature) large staphylococcus-like bodies were seen; they were probably contaminations, and were not investigated further.

On the other hand, in the cultures in blood-serum at laboratory-temperature the parasites went through the development described in detail below; they increase in size, multiply greatly, and finally become *Herpetomonas*-like flagellates.

(2) The parasites in the sore.—In smears from the juice of the sore stained with Giemsa's stain the parasites are seen in all sorts of shapes—pear-shaped, oval, torpedo-shaped, and even spherical (figs. 1, 2, *a-f*, 3, *a-g*). They are found free outside the corpuscles and also in the large macrophages, in some of which they may occur in considerable numbers (fig. 1). The individual parasites consist of faintly staining protoplasm with macronucleus and micronucleus in various forms. The parasites appear to have a distinct capsule,

shown as a well-defined margin. The length of the body is about one-third that of a red blood-cell; the parasites are therefore a little smaller than those described by James (2), but on the whole the descriptions of Wright (3) and James are applicable to the bodies seen in my specimens.

The micronucleus seems to be more often dot-shaped than rod-shaped, and separation of the dot-shaped micronucleus from the macronucleus can be seen in different stages (figs. 2 and 3). From the study of the smears I am inclined to think that the youngest stage of the parasite is the torpedo-shaped form (fig. 2, *a-c*) with a single nucleus [or with the two nuclei closely apposed], and that the separation of the two nuclei in these spore-like forms comes about after the parasite has entered the macrophage; in other words, that the parasite undergoes development in the macrophage, and that the free parasites showing the typical characters of the two nuclei (fig. 2, *d, e*, fig. 3, *d, f*) are those which have been liberated from a macrophage, either by its disintegration or by ejection from it.

The parasites contained in macrophages are seen to be lodged inside a vacuole or clear space (fig. 1); possibly some sort of secretion is thrown out round the parasite by the macrophage.

The parasites multiply by fission (fig. 3, *e, g*), as described by previous observers. Heart-shaped nuclei are seen in some of the parasites in the smears.

(3) The phases of the development of the parasite in cultures.—After twenty-four hours the cultures, examined in the fresh condition or in stained smears, showed no very obvious increase in the numbers of the parasites, but a sparing distribution in groups of four or eight. The body of the parasite is increased to double its former size, but shows no other difference, except, perhaps, rather better staining of the body-protoplasm.

After forty-eight hours the parasites have grown and multiplied enormously, and are found in masses (fig. 4), visible under the low power. Isolated individuals are also to

be found with high powers, but these are obviously separated from the colonies in the preparation of the smear. The masses consist of great numbers of parasites growing in colonies and entwined with one another. The colonies may be compact and more or less spherical, in which case it is more difficult to make out the shape of the individual parasites; or they may be looser in texture with the individuals more separate (fig. 4).

The parasites have now increased in length to two and a half or three times the diameter of a red blood-corpuscle, and in breadth to about half or two-thirds this diameter. The shape of each individual is like a banana (fig. 4); the contour of the body is well defined, the sides being parallel and the ends rounded; the anterior extremity bearing the micronucleus is more rounded than the other. The parasites are not mobile or amœboid, and for the most part no flagellum can be detected, though in some this organ is already present (fig. 5, *a-c*). The body-protoplasm shows a slightly spongy character, and takes up a deeper stain than in younger forms. The macronucleus is centrally situated and is less compact than in young forms. The micronucleus has shifted to one end of the body and shows a faint semblance of a vacuole round it.

At seventy-two hours the development is complete (fig. 6, *a-j*). The flagellum seems to be formed a few hours after the forty-eight-hour stage as an outgrowth from the micronucleus of a fine filament, which probably shoots out rapidly at the stage when the parasite may be supposed to have reached maturity. Once the flagellum is formed, the individual is liberated and is free to swim away from the colony. Here and there groups of six or more fully developed flagellated individuals are found, entangled by their intertwining flagella.

The body of the parasite is practically the same as described at forty-eight hours, except that it has grown a little longer and stouter, and the distinction between the posterior pointed and the anterior flagellate end is better marked. The flagel-

lum is one and a half times or twice the length of the body of the parasite, and has from six to eight symmetrical undulations. In life the flagellum is very active, and moves very rapidly with a lashing, wave-like, not a corkscrew-like movement; in appearance it strongly resembles a spirochæte.

The parasites are frequently found in pairs (fig. 6, *f-j*) [doubtless indicating fission]. Sometimes a pair consists of a thinner and a thicker individual [compare the splitting off of spirillar forms in *L. donovani*, described by Leishman and Statham, 'Journ. R.A.M.C.,' iv, 1905, p. 321].

[The foregoing account contains the pith of Dr. Row's first letter to me, and appears to represent the normal healthy development of the parasite in cultures. With the object of obtaining a slower development of the parasite, especially of the stages previous to the formation of the flagellum, material was taken from a case of longer standing, and the parasites were allowed to "stew in their own juice" for three days in a sealed tube at laboratory temperature before cultivating them. The result was a slower development of the parasites in the cultures, with production of peculiar forms probably representing abnormal or degenerative forms of the parasites weakened by the unfavourable conditions of its development. An account of these cultures forms the substance of Dr. Row's second communication to me, and a brief abstract of it follows.—E. A. M.]

The parasite, when derived from an old case (in which the lesion is about to break down into pus and is on the point of ulcerating, and when, consequently, the contents of the lesion are rich in leucocytes and pus), gives rise in cultures to a slow and irregular development, both in numbers and in morphological characters. Under these unfavourable developmental conditions very few typical well-developed and mature flagellates are produced; the products of stunted forms either do not reach the flagellate stage at all, or, if they do so, they are unable to continue their existence long.

The following seems to be the general plan of the development. The parasites begin to elongate into ovoids (fig. 7, *a*)

and then become pear-shaped (fig. 7, *b-h*), with one end pointed. The nucleus divides and division of the body follows (fig. 7, *j-l*). The pear-shaped parasite thus gives rise by fission to a slender form and a stout form (fig. 8, *a*). Each of these divides again twice (fig. 8, *b-i*), so that from one parasite are derived eight flagellates—four long forms and four short and stunted forms (compare fig. 8, *l*). The stunted forms are short-lived, but the long forms persist in cultures of even 120 hours' standing. All the division takes place before the flagellum is formed; after this event there seems to be no further multiplication. [This conclusion can only apply to the stunted cultures; in the more healthy cultures, as stated above, there is evidence of multiplication of the flagellated forms. Moreover, the vast masses of parasites in the forty-eight-hour healthy cultures indicate that here many more than eight flagellates are derived from a single Wright's body.]

ADDENDUM.

[When this memoir was completed and ready to send away I received, on March 27th, a third communication from Dr. Row, containing in concise form his conclusions, which I append here in his own words. Dr. Row exhibited his preparations and read a paper on them at the Medical Congress held in Bombay in February of this year.—E. A. M.]

I conclude that the parasite of the oriental sore (*Leishmania tropica*) and that of kala-azar (*L. donovani*), although apparently identical when examined in smears direct from the lesion, are distinct when examined in cultures, and for the following reasons:

(1) The parasite of oriental sore, when fully developed into the flagellate forms under ordinary conditions of culture, is much longer and bigger than that of kala-azar, where one meets with shorter and stouter forms, as a rule.

(2) The parasite of oriental sore is more resistant to external conditions than that of kala-azar; in other words it

is much less delicate, as it is possible to obtain developmental forms up to the flagellate stage from the parasite of oriental sore three days after its removal from the lesion, while the parasite of kala-azar, according to Rogers, dies within twenty-four hours after it leaves the spleen, if not cultured within that period.

(3) The flagellum of the parasite of oriental sore is much longer, and presents more regular wavy undulations than that of kala-azar, where it is shorter with less regular undulations.

(4) Although contamination of the material with extraneous germs is inhibitory to the early developmental progress of the parasite of oriental sore, it is not so destructive to its further development into flagellates as in the case of the parasite of kala-azar, where, according to Rogers, the slightest contamination with staphylococci is sufficient to destroy the culture.

(5) The parasite of oriental sore develops into fully mature flagellate forms between forty-eight and seventy-two hours, while that of kala-azar takes twice as long if not longer.

(6) The best culture medium (according to my results) for the parasite of oriental sore is human blood-serum, by preference that from tuberculous patients; while that for the parasite of kala-azar, according to Rogers, is sodium citrate, 2–10 per cent., in sodium chloride solution 0·8 per cent., mixed with splenic blood.

(7) The optimum temperature for the growth of the parasite of oriental sore is between 25° and 28° C., or even up to 30° C., while for the parasite of kala-azar it is 22° C., or even less, according to Rogers.

[Postscript (July 5th, 1909).—Since the above was sent to press I have been informed by Sir W. B. Leishman that the parasites of oriental sore have also been cultivated by Nicolle, whose memoir, however, I have not been able to see.—E.A.M.]

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EXPLANATION OF PLATE 20,

Illustrating Dr. Row's paper on “The Development of the Parasite of Oriental Sore in Cultures.”

[All figures are drawn with the camera lucida to a magnification of 2000 diameters from preparations stained with Giemsa's stain.]

FIG. 1.—Large macrophage containing numerous parasites, from a smear of the juice of the sore.

FIG. 2, *a-f*.—Free parasites from the same smear; *a-c*, young forms with micronucleus and macronucleus closely apposed; *d-f*, parasites probably set free from a macrophage.

FIG. 3, *a-g*.—Free parasites from a smear of an old case, showing various shapes of the body and conditions of the nucleus; *e, g*, dividing forms.

FIG. 4.—Mass of parasites from a smear of a culture of forty-eight hours' standing, showing various shapes and sizes of the parasite.

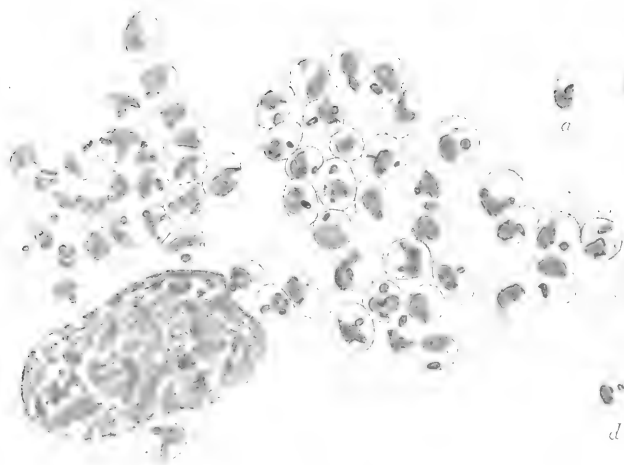
FIG. 5, *a-c*.—Three flagellated individuals from the same preparation as the last figure.

FIG. 6, *a-i*.—Flagellated *Herpetomonas*-forms from a smear of a culture of seventy-two hours' standing; *a-e*, various forms of single flagellates; *f-i*, parasites in groups and pairs.

FIG. 7, *a-o*.—Parasites from a smear of a retarded culture of twenty-four hours' standing; various stages of the development are seen. Some parasites, such as the two small ones in *g*, have not developed at all; others, such as *k* and *l*, have developed much further and are dividing.

FIG. 8, *a-m*.—Parasites of a smear of a retarded culture of forty-eight hours standing. *a*, division into a stout and a slender form; *b-d*, division of stout forms; *e-i*, division of slender forms; *j, k*, groups showing various stages of development; in *j* is seen a parasite not advanced beyond the initial stage; *l*, a group showing a pair of slender forms, recently divided off, and a pair of stout forms in the act of dividing; *m*, two pairs of slender forms (one dividing) with flagella growing out.

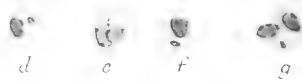
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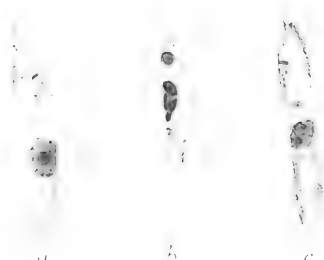
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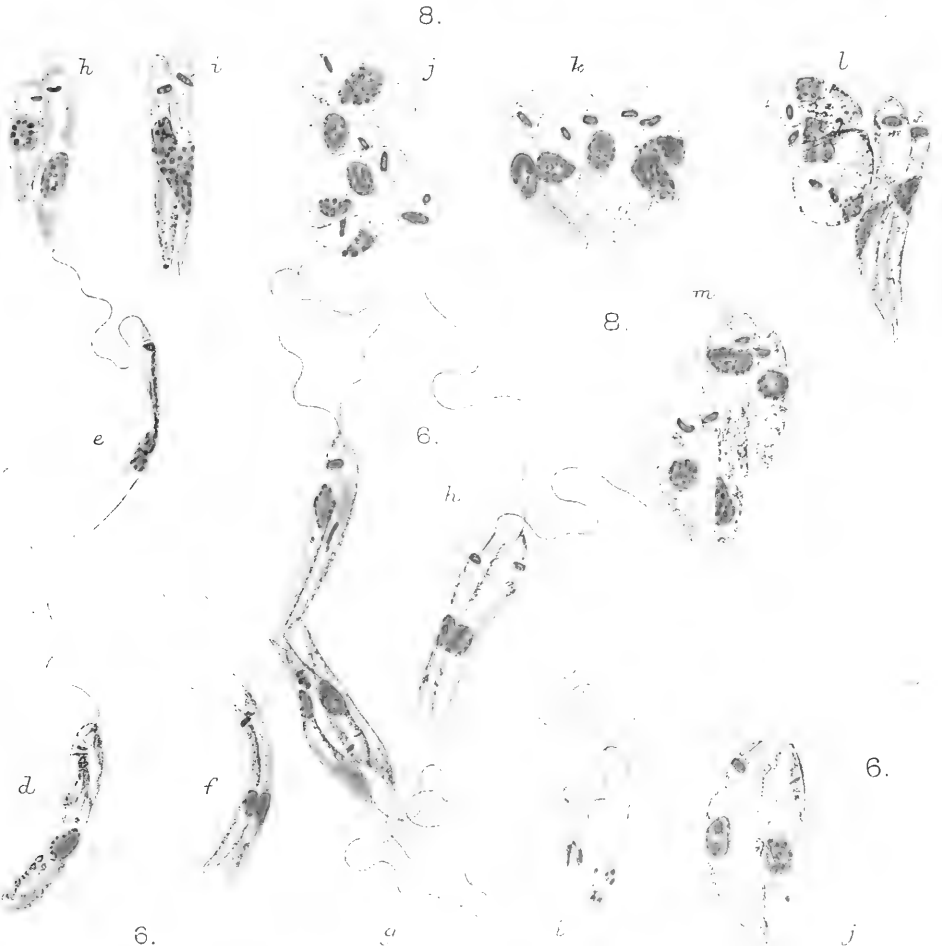
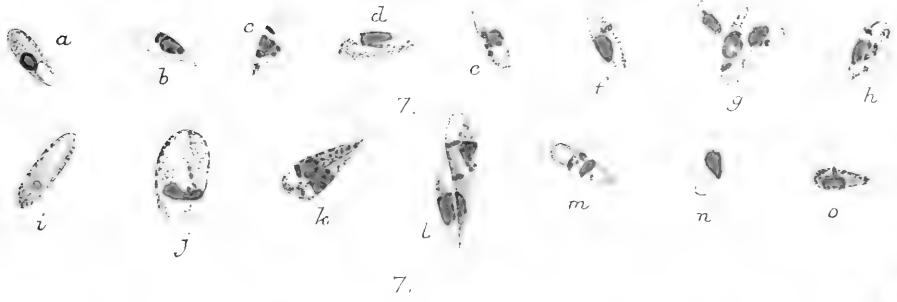


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5.







The Structure of *Trypanosoma lewisi* in Relation to Microscopical Technique.

By

E. A. Minchin,

Professor of Protozoology in the University of London.

With Plates 21, 22, and 23.

INTRODUCTION.

MUCH has been written of late years about the minute structure of trypanosomes, and also about the merits or demerits of the various methods in vogue for preparing them for examination with the microscope, that is to say, the technique of fixing, staining, and preserving these tiny creatures. Since our knowledge of the structure of trypanosomes is based almost entirely on the results of a number of complicated chemical and physical processes practised on a very delicate protoplasmic body, it is clear that a knowledge of the effects produced by these processes on the organisms are most important in interpreting the microscopic image finally obtained, in order to estimate, in any given case, how far the trypanosome may have undergone deformation or change as the result of the treatment it has gone through. In a perfect state of scientific knowledge it would, no doubt, be possible to deduce exactly such results from the known action of the reagents employed upon the protoplasmic body, but in the present condition of our knowledge it is only possible to arrive empirically at an approximate estimate of the effects of technique by comparing carefully the results yielded by it.

In order to eliminate as much as possible disturbing factors

due to variability in the objects themselves, I have made use in these researches of the common trypanosome of the rat, since it can be obtained in a practically monomorphic condition and does not exhibit the remarkable polymorphism shown, for example, by the trypanosome of sleeping sickness. Moreover, after about the tenth day of infection all multiplication ceases, and though individuals may be found occasionally with two or even three trophonuclei, I am convinced that this condition has nothing to do with division, as has sometimes been supposed, but is to be regarded as an abnormality. Hence in *Trypanosoma lewisi*, after the multiplication-period is over, the variability in size and structure is reduced to a minimum, and the details of cytological structure are not complicated by changes due to processes of fission or multiplication.

This memoir is divided, therefore, into two parts. In the first part I shall deal with the subject from the point of view of the technique, and discuss the results obtained by different methods of fixing, staining, etc., under their respective headings. In the second part I shall discuss the structure of the trypanosome itself, taking its various parts in order. In dealing with two subjects which, though distinct, necessarily overlap to a certain extent, it is very difficult to avoid repetition, and though I shall try to steer clear of this defect in exposition as much as possible, I must crave indulgence in those parts of my subject where repetition is an alternative to omission of necessary statements.

PART I.—TECHNIQUE.

The subject of modern microscopical technique is an absolutely inexhaustible study; no one in a human life-time could claim to have said the last word upon it. As I write, all sorts of methods that I have not tried, or variations upon methods that I have tried, present themselves to my mind. It becomes necessary in a research of this kind to set a term to one's work, and to rest content at a certain period with what one has achieved, however incomplete it

may appear to oneself or to others. Circumstances, into which I need not enter, oblige me to break off at the point I have reached, and to bring forward my results, such as they are, without undertaking further investigation.

The object of microscopic technique when applied to an organism such as a trypanosome is to produce a microscopic image which shall represent, so far as possible, the form and minute structure which we believe the organism to possess actually in the living state, true in all details, exact to a definite known scale of magnification, and coloured artificially so as to assist in rendering visible the different parts of the organism. A method which would have this ideal result would be a perfect method; but unfortunately no such method is known to exist, since all technique deforms or falsifies the form or structure of the organism to a greater or less extent. We are therefore confronted at the start with the difficulty, that since we can only arrive at a conception of the true and actual structure of the objects by the use of methods of technique, we are obliged to estimate the deviations from the truth, produced by technique, solely by a comparison of results which are themselves one and all defective. The only conceivable method by which the true form and structure of *Trypanosoma lewisi* could be recorded would be by a photograph of it in the living state; but I know of no method by which a snapshot can be taken of a minute and transparent organism, in a state of incessant and active movement, at a magnification of 2000 or 3000 diameters.

It was necessary, therefore, to find at the outset some method of killing and preserving the trypanosomes with the least possible deformation or alteration, in order to serve as a standard of comparison for other methods. It has always been my experience, and I think that of others, that in the case of Protozoa of larger size, such as Ciliata, Amœbæ, etc., the most life-like preparations can be obtained by simply exposing suddenly the living organisms, moving freely in a small drop of the medium they inhabit, to the vapour of strong osmic acid. The organisms are then examined without further

treatment, except that the preparation is sealed up to avoid evaporation of the fluid medium, and in this way it is possible to obtain very perfect temporary preparations, which can be kept unaltered for at least some months. I made use, therefore, of this method for trypanosomes, but on account of the medium, blood, in which they live, special precautions were necessary in the application of it.

My first method was to take an ordinary microscope slide with a small depression hollowed out in the centre, and to cement on to this a ground-glass ring, surrounding the hollow. The upper edge of the ring was then painted with vaseline, and a drop or two of osmic acid solution (4 per cent.) placed in the hollow of the slide. Now a coverslip was taken and a drop of fresh blood placed in the middle of it and spread out with a clean glass rod, not, however, smeared out into a thin layer. It was then placed with the blood downwards on the ground-glass ring over the osmic acid, and the coverslip pressed down all round on the vaseline. All this manipulation was done with the greatest possible rapidity, in order to avoid evaporation of the blood as much as possible. Thus the blood in a hanging drop is exposed to the vapour of strong osmic acid in an air-tight chamber; it is, of course, important that the blood should not come into contact with the osmic acid solution. It is now possible to examine the trypanosomes without further treatment; but owing to the thickness of the preparation, difficulties arise in the illumination of the object, since it is not possible to focus the sub-stage condenser properly for the use of the highest powers. I was able, however, to draw the trypanosomes in outline, at a magnification of 3000 (figs. 1, 2), but could not make out minute details. In order to do this a further manipulation was necessary.

After the coverslip with the blood had been exposed to the osmic vapour for a certain time, it was picked off the glass ring and at once placed with the blood downwards on an ordinary clean slide. From the glass ring the coverslip takes with it a ring of vaseline, which when pressed down on the

slide forms an air-tight cell for the enclosed blood. To ensure against the evaporation the coverslip is further luted all round the edge.¹ The blood is thus removed from the osmic chamber with no other alteration than the amount of evaporation which it suffers during the rapid passage through the air from the glass ring to the clean slide. Preparations made in this way keep without perceptible alteration for months; the trypanosomes can be examined with any power of the microscope and drawn to any scale; it is possible to make out all details of structure in them and even some which cannot be made out in other ways (figs. 3-8). The trypanosomes have undergone no changes during the treatment than such as result from the fixation with the vapour of osmic acid on the one hand, and such as may be due, on the other hand, to the slight amount of evaporation of the blood-serum which is inevitable, however rapidly the manipulation is carried out; hence these preparations represent, in my opinion, the nearest approach possible, in the present state of our technique, to the living condition, and may therefore serve as a standard for comparison with the results of other methods. I shall therefore refer briefly in the sequel to these preparations as "the standard," whenever I have to speak of them.

In making the standard preparations I introduced one additional complication into some of them, namely, that on the clean slide a small drop of acidulated methyl-green solution was placed ($\frac{1}{2}$ per cent. methyl-green in 1 per cent. acetic acid), and the blood, after exposure to the osmic vapour, was placed on the stain. In the course of a few hours the stain mixes with the blood and tinges the nuclei of the trypanosomes. Comparison of trypanosomes, treated with the acidulated methyl-green (figs. 5-8), with those in which no stain was used (figs. 3, 4), revealed no perceptible difference in size,

¹ I employ for this Czokor's mixture of beeswax and Venetian turpentine, heated together until the mass is hard and smooth (not sticky) when cool, and applied to the edge of the coverslip with a piece of heated wire.

form or general structure,¹ so that no ill-effects result from the use of the stain, while the microscopic image is rendered much sharper and clearer.

My method of carrying out investigations upon the effects of technique was the following: Preparations of *Trypanosoma lewisi* were fixed and stained in various suitable ways, and the results were all drawn with the camera lucida at a magnification estimated at 3000 diameters, and are reproduced in this memoir at the same scale. It is possible that the figure 3000 is not perfectly accurate, but if not, it makes no difference to the result, since the object was to compare the effects of the methods employed, and for purposes of comparison the exact magnification is immaterial, provided it be uniform throughout. All the drawings given here were executed using always the same microscope and length of tube, the same lenses, camera lucida, drawing board and illumination. Some of them were done by me, but most of them by my assistant, Miss Rhodes, to whom my best thanks are due for her skilful help. Zeiss's apochromatic objective 2 mm., 1.40 aperture, was used, combined with compensating oculars. The source of illumination was the flame of an oil lamp placed edgewise, and concentrated by a Zeiss collecting lens, through a monochromatic screen on to the mirror of the microscope, and thence reflected through a centring achromatic condenser of Zeiss. This illumination, if properly arranged and focussed, gives very perfect definition—a very important point in studying these preparations; I have frequently found that structures had been overlooked which became visible with improved illumination. I frequently compared the results obtained in this way with those given by a monochromatic illuminating apparatus set up with a prism in such a way that any part of the spectrum could be used, in order to obtain confirmation of the structures as drawn with the illumination described.

¹ A slight vacuolation, which is probably an artefact, makes its appearance near the kinetonucleus after treatment with methyl-green (figs. 5-8).

Before proceeding to describe in detail the results of the fixatives and stains used by me, I will say a few words about the methods of applying them. I began with the ordinary method of making smears on slides; this is quite suitable for smears that are to be dried off or fixed with osmic acid vapour, for which the procedure adopted was the following: a glass tube is taken, of suitable size and calibre, and provided with a tightly fitting cork or stopper; at the bottom of the vessel are put twenty drops of 4 per cent. osmic acid solution with one drop of glacial acetic; when the smear has been made, the slide bearing it is placed with as little delay as possible into the tube containing the osmic acid and corked up. It is advisable to take precautions against any part of the blood-smear coming into contact with the osmic acid solution, if one wishes to be economical in the use of this most expensive re-agent. An exposure of thirty to forty-five seconds to the osmic vapour is sufficient; the fixation is practically instantaneous. Slides fixed with osmic vapour in this way may be (1) dried off, then fixed with absolute alcohol or methyl alcohol in the ordinary way; or (2) placed at once without drying into absolute alcohol, stained as desired and then dried off; or (3) fixed, stained, and mounted in Canada-balsam without ever having been dried.

I soon, however, abandoned the use of slides for smears for various reasons. In the first place it is a clumsy method for rapid manipulation, and however anxious one may be to shorten the exposure to the free air, it is very difficult to avoid a certain amount of drying taking place in such large smears, either at the edge or in thin places. In the second place, slide smears do not give good results when the fresh wet smear has to be fixed by sudden immersion in a liquid, which it almost necessarily enters with a dive, so to speak, with one end foremost; in such cases it is common to find all the blood-corpuscles drawn out in an extraordinary manner, and all the trypanosomes stretched out straight in one direction, indicating the line of the dive into the fixative. In the third place I may point out that since the best immersion objectives are

corrected for coverslips, naked smears without coverslips do not give optical results so good as those covered, and that in covered smears it is optically preferable for the smear to be on the coverslip rather than on the slide.

The majority of the preparations described here were done by Schaudinn's method of making smears on a coverslip and then dropping the coverslip flat down plump into the fixative. I hold a coverslip in the fingers of my left hand, a glass rod in my right; in front of me is the liquid to be used for fixation. An assistant draws the drop of blood from a rat's tail, and either places it on the coverslip I am holding, or I take it up on the end of the glass rod from the tail; in either case I smear the blood out with the glass rod and drop the coverslip, with the smear downwards, into the fixative. The whole process is done with one turn of the right wrist and one of the left, far more rapidly than a slide can be manipulated and with much less chance of drying or time for evaporation to take place. Coverslip smears made in this way are always stained and mounted in Canada-balsam without being allowed to dry during any part of the process. They are most conveniently handled in solid watch-glasses (that is to say, a square slab of plate glass, two and a half inches square and a quarter of an inch in thickness, hollowed out on one side into a cavity the size of a watch-glass); the coverslip can be placed in this with only sufficient liquid to keep it wet on the underside (the side of the smear), and can be examined under the microscope with moderate magnification at any time, and the glasses can be stacked one on the top of the other to prevent the contents drying up.

The use of coverslip smears necessitates a modification in the mode of applying the osmic acid vapour for fixation of films. I take a square block of hard paraffin of suitable size, and a glass ring ground flat on the two sides. The glass ring is gently heated and so cemented to the middle of one surface of the block of paraffin, which is then hollowed out into a small cell inside the glass ring. The osmic acid is placed in the cell in the paraffin, and the coverslip, with the blood-smear

downwards, is placed on the glass ring, to which it sticks by means of the wet blood itself, and makes a practically airtight chamber. A certain amount of the smear has to be sacrificed, of course, by this method, but there is always enough for all practical purposes preserved within the ring. After sufficient exposure the coverslip is lifted carefully off the ring and dropped at once into absolute alcohol or other fixative. In this method the only precaution necessary is the protection of the operator's eyes and air-passages from the vapour of the osmic acid. It is advisable either to wear protective spectacles or to have the osmic cell covered with a glass plate, which an assistant removes the instant it is desired to place a coverslip on it.

Salvin-Moore and Breinl (1907) recommend making smears on slides previously covered with a thin layer of glycerine and albumen, but with what object I do not understand, as the blood-films always stick on perfectly well to the coverslips and slides when plunged suddenly into fixatives, and neither corpuscles nor trypanosomes come off. Even when working with fish-blood, in which the parasites are exceedingly scanty, I have always found them in my never-dried smears just as abundantly as in those done by other methods. In our technique the frail bodies of these unfortunate creatures are subjected to so many processes of violent treatment that to avoid deformation it is better to reduce and eliminate these processes as much as possible rather than to increase them. The addition of glycerine and albumen to the fresh blood containing living trypanosomes can but be an additional source of error, and is therefore, in my opinion, best avoided.

I made several attempts to obtain preparations of trypanosomes by the method recommended by Schaudinn (1902, p. 190) for malarial parasites, that is to say by dropping fresh-drawn blood into fixatives, such as Flemming's fluid or Schaudinn's fluid, in a centrifuge tube, and then carrying out all the subsequent processes of washing, staining, etc., by means of the centrifuge. After much searching I found some

trypanosomes in preparations made in this way, but they were so shrunk and deformed as to be scarcely recognisable.

When preparations that have been mounted in Canada-balsam without being dried at any time are compared with those that have been dried off at some stage of the process, either before fixation, or after fixation with osmic vapour, or after staining, it is found that the trypanosomes in the never-dried preparations are constantly slightly smaller than in the dried-off preparations. Comparison with the standard shows that the dried-off trypanosomes have lost very little in bulk, while the never-dried trypanosomes have lost considerably. I obtained invariably the same results with the trypanosomes and trypanoplasms of fishes; the never-dried specimens mounted in Canada-balsam were always a size smaller than those in the dried-off films. This result proves, in my opinion, that the changes of medium which are incidental to the processes of dehydration with alcohol, clearing with xylol or other media, and mounting in Canada-balsam, have the effect of diminishing the size of the body. So long as it is kept in a semi-fluid plastic condition the body is susceptible to changes of medium, and can be shrunk or swollen by them. If, however, all fluid be once removed from the body by evaporation, it appears to obtain a rigidity of texture which resists further deformation, although it may be altered in form to a greater or less extent by the process of drying itself.

After these general remarks I proceed to describe in detail the effects produced on *Trypanosoma lewisi* by various fixing and staining methods, using as the standard of comparison the preparations fixed simply with osmic acid vapour and examined in the fluid blood, as described above.

(1) Fixatives.

The fixatives I have used may be grouped conveniently under the following heads: (1) Osmic acid vapour, followed simply by alcohol; (2) mixtures containing osmic acid, (3) mixtures in which corrosive sublimate is the principal in-

gradient; (2) and (3) were either used directly on the wet film, or after previous exposure to osmic acid vapour.

(1) Osmic acid.—I have employed osmic acid as a fixative of the films followed by various other fixatives, such as absolute alcohol, Flemming's fluid, sublimate-acetic, etc. I will only deal in this section with its action when followed by alcohol, and discuss its action when combined with other fixatives in the sections dealing with those fixatives. Of all the methods I have tried, fixation with osmic vapour followed by fixation with alcohol (figs. 60–71) gives the best result as regards size, form, and general characters of the trypanosomes, so far as can be judged from a comparison with the standard (figs. 1–8). The osmic acid was used in the form of vapour given off from a 4 per cent. solution, combined with acetic acid in the following proportions: a 4 per cent. solution osmic acid, 20 drops, glacial acetic acid, 1 drop.

The smears, after exposure to the osmic vapour, were in some cases allowed to dry off, either before further fixation or after staining; but as a rule they were transferred at once to absolute alcohol, and were stained and mounted in Canada-balsam without being allowed to dry at all. I will distinguish these two methods of treatment briefly as the dried-off osmic method and the never-dried osmic method respectively. The dried-off method is most convenient for smears made on slides; for the never-dried methods it is better to make the smears on coverslips. The dried-off osmic smears stain very well by the ordinary Romanowsky methods (Giemsa's stain, azure-erythrosin, etc.), but I have never got good results with the iron-hæmatoxylin stain after drying off. The never-dried smears stain well with Romanowsky stain, and fairly well, but rather faintly, with iron-hæmatoxylin. The details of the flagellum and blepharoplast are difficult to make out after osmic-absolute fixation and iron-hæmatoxylin staining; for these points fixation in sublimate mixtures is far superior.

After the dried-off osmic method the trypanosomes do not show what I will term briefly a "periplast-line," that is to say, a clear border under the periplast, causing the periplast

to stand out distinctly from the body (see below). By the never-dried method, however, a periplast-line appears very distinctly in the iron-hæmatoxylin preparations (Pl. 21, figs. 10, 11). Its appearance in the preparations stained with the Romanowsky stain depends largely on the degree to which the stain has been extracted by the acetone during the process of mounting in Canada-balsam, and the result varies greatly in one and the same slide (Pl. 22, figs. 64-69); a matter which I shall discuss more fully in the section dealing with this stain and its effects. One of the most constant results of the process of drying off is seen in the relations of flagellum, blepharoplast and kinetonucleus. In never-dried preparations the flagellum starts from close to the kinetonucleus, with which the blepharoplast is nearly in contact. In dried-off preparations, on the other hand, there is a distinct interval between blepharoplast and kinetonucleus, almost equal to the width of the latter in many cases (Pl. 22, figs. 60, 61). The same result is seen in preparations in which osmic fixation is followed by Flemming's fluid or other reagents, and dried off after staining with Romanowsky's stain, and also in preparations dried before fixation. It follows, therefore, that the effect of drying is to draw apart kinetonucleus and blepharoplast, probably as the result of a longitudinal contraction in the flagellum or in its basal portion.

(2) Osmic acid mixtures.—The two famous combinations, Flemming's fluid and Hermann's fluid, were used, the former both direct on the wet film and after previous exposure to osmic vapour, the latter only directly on the film. The Flemming's fluid used was the so-called strong mixture; the Hermann's fluid was made up in the same manner, and differed only in the substitution of platinum chloride (so-called), 1 per cent., for the chromic acid, 1 per cent. A variety of staining methods was tried on these films; I will describe the results obtained by the various stains in the sections dealing specially with them below. The best results were obtained with iron-hæmatoxylin, and the following account of the action of these two fixatives refers more particularly to

preparations stained by this method, unless the contrary is stated.

When used directly on the wet films both fixatives gave very similar results. The trypanosomes are under-sized and deformed, appearing too short in proportion to their breadth, as if contracted in the longitudinal direction (figs. 36-38, 40-44); their attitudes in the preparations look strained and unnatural, as if their end had not been a peaceful one. The amount of the deformation is distinctly greater after Hermann's fluid than after Flemming's, and with the latter re-agent it varies in different preparations. I am inclined to think that the amount of deformation is related in an inverse manner to the degree to which the films have lost moisture previous to fixation. However carefully and rapidly the preparation of the film be carried out, it is clear that a thin film of blood must lose a certain amount of moisture before it reaches the fixative. My impression is that the more the film dries before coming into the fixative, the less the trypanosomes undergo shrinkage and distortion of the body-form.

As regards minuter details, the following points are to be noted: The body of the trypanosome does not show a periplast-line; the nucleus has an appearance most characteristic of the effects of these fixatives; it is surrounded by a clear space, often very distinct, but not sharply limited (figs. 36-38, 43, 44), which I regard as the result of shrinkage. In the nucleus the karyosome is very distinct, and frequently also other coarse chromatic granules are to be seen. A similar condition of the nucleus could be made out also in trypanosomes stained with all the other staining methods that were used, and must, therefore, be attributed to the action of the fixative.

The kinetonucleus and blepharoplast appear normal after Flemming's and Hermann's fluids, but the flagellum often appears extremely crinkled, with sharp bends and angles, very different from the normal smooth undulating curves; its appearance is strongly suggestive of longitudinal shrinkage.

In extreme cases (Pl. 21, fig. 38) the appearance of the flagellum recalls that of a wire that has been untwisted from the neck of a soda-water bottle.

In films fixed with osmic acid vapour before being put into Flemming's fluid, I find but little difference from those fixed with absolute alcohol after the osmic treatment, except that the trypanosomes are rather broader in proportion to their length (figs. 80, 81). The nucleus stained with Giemsa's stain is a large red patch, as large as, or even larger than, the whole clear space seen after direct fixation with Flemming's and Hermann's fluids. When stained with iron-hæmatoxylin, after Flemming's fluid preceded by exposure to osmic acid vapour (fig. 39), the nucleus of the trypanosome does not show the clear space round it described above, but appears as a broad oval or nearly circular in outline. The body shows no periplast-line and the flagellum has the normal appearance. Previous exposure to osmic acid vapour would appear, therefore, to obviate some of the defective results obtained by the direct action of Flemming's fluid.

(3) Sublimate mixtures.—Three different sublimate mixtures were used for fixation: (1) Sublimate and acetic acid, (2) Schaudinn's fluid, (3) Mann's picro-corrosive mixture. All three were used directly on the wet film. The first two were used also after previous exposure of the wet film to osmic vapour. In all cases the fixing solution was allowed to act for about half an hour on the film.

(a) Sublimate acetic.—This was used in the proportion of 95 parts (by volume) of corrosive sublimate solution, saturated in distilled water, and 5 parts of glacial acetic acid. A mixture was also tried of 99 parts corrosive sublimate solution to 1 part of glacial acetic, but was found to produce very marked shrinkage in the trypanosomes (Pl. 21, figs. 12, 13) and was not used again.

The fixative was used either directly on the films (figs. 19–22, 82–84, 88–91), or after previous exposure to osmic vapour (figs. 14–18, 75), in both cases without drying before putting in the sublimate solution. The fixed films were stained in

various ways, principally with the Romanowsky stain, with iron-hæmatoxylin, and with Twort's stain. Some of the films stained by the Romanowsky method were dried off (Pl. 22, figs. 70, 73), but as a rule they were kept wet and passed through acetone and xylol into balsam. All films stained with iron-hæmatoxylin or with Twort's stain were kept wet throughout and mounted in balsam.

After previous fixation with osmic vapour the trypanosomes generally show a distinct periplast line (figs. 17, 18, 75), not to be seen when the sublimate mixture is used directly. There is little other difference to be noticed between osmic-fixed films and those in which osmic has not been used. With either method iron-hæmatoxylin gives good results; Twort's stain, however, does not work well after osmic fixation, but stains very well after fixation direct in the sublimate mixture (figs. 82-84).

(b) Schaudinn's fluid (two volumes of saturated solution of corrosive sublimate in water, one volume of absolute alcohol, with the addition of a few drops of glacial acetic).—This fluid was also used with (figs. 23, 24) or without (figs. 25-28, 76, 85) previous exposure of the film to osmic vapour. The films were never dried and were mounted after staining in Canada-balsam. The results were on the whole similar to those observed with sublimate acetic. A periplast-line can generally be observed, and appears to depend on the degree of extraction of the stain, as is well seen after iron-hæmatoxylin (figs. 23-38). Twort's stain gives very good results if the fixing fluid be used without previous osmic vapour-fixation (fig. 85).

(c) Mann's picro-corrosive.—Made up as follows: 2.5 grm. of corrosive sublimate dissolved in 100 c.c. of boiling distilled water; when dissolved, 1 grm. of picric acid added; the mixture allowed to cool, and either used as made up, or with addition of 15-20 c.c. of formol, mixed in immediately before use. Wet films were fixed in the mixture and stained in various ways without drying at any time. I could perceive very little difference resulting from the presence or

absence of formol, but got the impression that the fixation was rather better, and the stain sharper, after use of the mixture containing formol.

Mann's fluid gave me very uniform results. The trypanosomes show no periplast-line and appear slender. The blepharoplast and flagellum show up with remarkable clearness, especially after iron-haematoxylin, and this appears to me the method of choice for the study of these structures.

With all the sublimate-containing mixtures that have been tried, a marked diminution of size is apparent in the never-dried preparations as compared with the type (compare especially figs. 1-8 with figs. 16, 35, and 79). The shrinkage is on the whole greatest after the picro-corrosive mixture; the disappearance of the periplast-line after fixation with picro-corrosive indicates, perhaps, a certain amount of collapse in the cytoplasmic body.

As already pointed out above, the shrinkage is scarcely perceptible if the trypanosomes have been allowed to dry off after exposure to the osmic vapour before fixation in the sublimate mixture. Figs. 70 and 73 show this plainly when compared with figs. 14, 15, 16, etc.

The facts indicate further that the shrinkage is due not only to the action of the fixative but also to the subsequent processes which the trypanosomes pass through, namely, first the staining process, secondly the dehydration and clearing preparatory to mounting in Canada-balsam. It can be observed, for example, that the Romanowsky stain, when used on trypanosomes which have not been dried, seems to shrink them more than do other stains, such as iron-haematoxylin or Twort's stain; compare, for instance, figs. 19, 20, 82-84 with figs. 21, 22, from a film which, after having been fixed and stained in Giemsa's stain without drying, was dried off after the stain; the trypanosomes are shrunk to a ludicrous extent in the latter case.

Although the trypanosomes are distinctly diminished in size and general proportions after sublimate fixation (when never dried), the shrinkage appears to take place evenly, and

the trypanosomes are scarcely if at all deformed. The body maintains an even, slender form, differing only from the type in size. The minute details of the nuclei, blepharoplast, and flagellum are very well seen after sublimate mixtures, and especially after picro-corrosive, better, indeed, than with any other fixative in my opinion.

(2) Staining Methods.

I have made trial of a number of staining methods on films preserved in various ways, and have obtained results which may be considered from two points of view. Some staining methods give good results generally, and yield what I may term "show" preparations — that is to say, preparations which one would not be ashamed to demonstrate to students or to strangers. Other stains give results which are quite useless considered as preparations for purposes of demonstration, but which may be quite interesting as micro-chemical reactions. I will discuss in detail the three staining methods that have given me the best results generally, namely the Romanowsky stain in its various modifications, Heidenhain's iron-hæmatoxylin method, and Twort's combination of neutral red and licht-grün; after which I will deal briefly with other stains I have tried.

(1) Romanowsky stain.¹—In applying this method I have always used Giemsa's modification; I obtain the stain made up in the fluid condition from Grüber, and for actual use I mix the stain with distilled water in the proportion of one drop of the stain to 1 c.c. of water. The preparations are left in from three to eighteen hours. Osmic-fixed preparations are apt to stain very darkly, and require a much shorter time than those simply fixed with alcohol. The preparations when taken out of the stain are washed with distilled water, treated for a few seconds with Unna's tannin-orange (obtained from

¹ I use the term "Romanowsky stain" as a general term for the combination of stains, of which the methods of Leishman, Giemsa, etc., are special modifications, in application or substance.

Grübler), and then washed in a current of tap-water for a minute or two. After this the preparation is washed with distilled water, and either dried off, or passed rapidly through three changes of acetone into xylol, and mounted in Canada-balsam.

Some preparations were also stained for me by Dr. J. D. Thomson with a mixture of azure and erythrosin in the following manner: A mixture was made of (1) azure I, 1 per cent., in equal parts of glycerine and methyl alcohol, 1 volume; (2) erythrosin, 0·1 per cent., in 0·25 per cent. formol, 2 volumes; (3) distilled water, 8 volumes. The two staining fluids, kept ready in solution, are mixed with the water immediately before use, and the mixture is allowed to act for from half an hour to one hour; it works better if kept at about blood-temperature. After staining, the preparations are either washed with distilled water only, or also with tannin-orange followed by tap-water, as described above, and then either dried off or mounted without drying in Canada-balsam, passing through acetone and xylol.

The azure-erythrosin mixture stains the trypanosomes in colours slightly different from that resulting from Giemsa's stain. The body is more purple in tint, and the red colour of the nucleus and flagellum appears rather deeper after azure-erythrosin. The general effects of the two methods are the same, and do not require to be discussed separately.

The Romanowsky stain is undoubtedly the best method for studying the general characters of the trypanosomes, especially if combined with osmic fixation, which, as I have shown, preserves very well the form and structure of the body and results in the minimum of shrinkage and deformation. I have always got the best results after osmic vapour followed by absolute alcohol; when the osmic is followed by other histological reagents, such as Flemming's fluid or sublimate-acetic, the results are inferior, but still usually quite good. On the other hand I have never got good preparations with the Romanowsky stain after wet fixation in hardening fluids without previous exposure to osmic vapour; the trypanosomes

become shrunk and deformed, often very greatly, and the stain is blotchy and irregular. That has been my experience in all cases with smears fixed in Flemming's fluid, sublimate-acetic, Schaudinn's fluid, Mann's fluid, etc., stained with Giemsa's stain, and mounted without drying in Canada-balsam or dried off. Preparations made in this manner are usually quite useless, while the companion slides, stained with iron-hæmatoxylin and mounted in the ordinary way, are very good.

The Romanowsky stain has the further advantage of being easy to apply and comparatively rapid in action. It is therefore the method of choice for workers to whom time, or laboratory equipment, are material considerations—that is to say, to those dealing in a limited time with large quantities of material, or working under disadvantages of installation, as in the tropics. Nevertheless I shall try to show that, as Schaudinn¹ has already stated clearly, the Romanowsky stain has some glaring defects in its action on minute structural details, and that some false conclusions and interpretations have been based upon its results. It is very important that its effects should be studied carefully and compared with those yielded by other methods, for only in this manner is it possible to eliminate errors and to discount false appearances produced by it, and to obtain, so to speak, a constant formula for estimating and interpreting the results of its use.

The peculiarities of the Romanowsky method of staining, as regards its effects on minute structures, are at once apparent when trypanosomes stained by this method are compared with others, fixed in precisely the same manner, but stained with iron-hæmatoxylin. The differences are very great, and often very puzzling; compare, for example, fig. 73, and figs. 14, 15; figs. 80, 81, and fig. 39; figs. 62, 63, and figs. 10, 11. To begin with the nuclear structures, the kinetonucleus appears

¹ "Nur ist sie (die Romanowsky'sche Färbung) mit Vorsicht zu benutzen, weil sie oft überfärbt und Strukturen vortäuscht, die garnicht vorhanden sind. Ohne Kontrolle durch Hämatoxylin darf man keine Schlüsse über Kernstrukturen bloss nach Romanowsky-Präparaten ziehen." Schaudinn (1902), p. 191.

much larger after the Romanowsky stain than it does after iron-hæmatoxylin, perhaps four times as large; the same two types of form are to be made out in the kinetonucleus, but they have become magnified, as it were, in the former case. The trophonucleus is not only much larger after staining by the Romanowsky method, but its structure appears quite different from that seen after iron-hæmatoxylin and other methods. In the place of a simple oval vesicle-like structure containing a deeply staining karyosome, we find after the Romanowsky stain an opaque mass of deeply stained coarse grains of reddish colour, in which a karyosome often cannot be made out with certainty. Sometimes the stained grains are closely packed and scarcely distinguishable individually; sometimes, and especially in preparations dried before fixation, the grains appear separate and distinct. Very frequently the nucleus shows a clearer spot at or near the centre (figs. 70, 80, 81). The flagellum appears thick and coarse and the blepharoplast is usually fairly large and distinct after the Romanowsky stain.

What is the explanation of these differences? The large size of the kinetonucleus and trophonucleus and the thickness of the flagellum after the Romanowsky stain are shown by the standard (figs. 1-8) to be a case of actual enlargement or thickening, not to be explained by supposing that after other methods, such as the iron-hæmatoxylin stain, these structures are shrunk. Such an enlargement can only be explained by a tendency of the red stain or stains, which are active in the Romanowsky combination, to deposit not only in, but also around, the structures that are coloured by them. Comparison of the kinetonucleus, in preparations stained by the Romanowsky method and by other methods, is alone sufficient to prove conclusively that the enlargement is due to this cause, and gives a clue to the interpretation of the very remarkable and perplexing differences seen in the structure and appearance of the trophonuclei in the two cases.

In order to discuss the effects of the Romanowsky stain on the trophonucleus, it is necessary that I should anticipate here

the conclusions to which I come further on in this memoir with regard to the minute structure of this body in *Trypanosoma lewisi*. The trophonucleus is a rounded oval body with a distinct limiting envelope, which is not to be regarded as a true nuclear membrane in the sense in which this term is used for metazoan nuclei, but, in all probability, as a condensation at the periphery of the chromatin-substance itself. Inside this envelope is a space, filled doubtless in the living animal by a nuclear sap, in which are contained other chromatin-bodies; first of all the conspicuous karyosome, sometimes double or further subdivided, which in its staining reactions resembles the kinetonucleus, and appears of dense texture; secondly, smaller granules of chromatin, some of which may be fairly large and plainly visible, but which for the most part appear to be minute and not capable of being resolved by the microscope as distinct structures, but are scattered in a state of fine division throughout the nuclear sap, giving to the trophonucleus the even dark grey tint which it shows in an iron-hæmatoxylin preparation at a certain degree of extraction, or the pale red tint which it exhibits after Twort's stain.

Applying the conclusion stated above, namely, that the red stain of the Romanowsky mixture deposits round the structures it stains, to the conception of the structure of the trophonucleus that I have put forward, we can explain the results of the stain as follows. The deposition of the stain round the nuclear membrane accounts for the apparent increase of size of the nucleus as a whole. At the same time the stain is deposited round the minute granules of chromatin within the nucleus, enlarging them to relatively coarse grains. The karyosome doubtless shares also in the enlargement, but is generally obscured by the closely packed and secondarily enlarged chromatin grains, and is consequently very difficult to make out clearly.

In support of the above conclusion I refer to the series of figures drawn from three preparations (*a*, *b*, *c*) which were made at the same time from the same rat, and which were all

fixed in the same manner, namely by osmic vapour followed by absolute alcohol, without drying at any time; *a* (figs. 64–69) and *b* (figs. 62, 63) were stained with Giemsa's stain, and passed through acetone and xylol into Canada-balsam; *c* (figs. 10, 11) was stained with iron-hæmatoxylin and mounted in the ordinary way.

In preparation *b* (figs. 62, 63) the passage through the acetone was evidently effected without any extraction of the stain taking place; the trypanosomes are perfectly similar in general appearance to those stained with Giemsa and dried off (figs. 60, 61). In preparation *a* (figs. 64–69), on the other hand, a considerable amount of extraction has taken place, which, however, is different in degree in different parts of the preparation; in one place a clump of trypanosomes may be found, all showing a deeply stained, opaque red trophonucleus; another patch of trypanosomes will show the stain greatly extracted. Hence in this preparation I have been able to find every stage of the extraction of the stain, and have put together the series shown in my figures.

The series begins with trypanosomes, in which the trophonucleus differs only from those in preparation *b* by its smaller size (fig. 64), showing that the process of extraction begins by removing the stain deposited outside the nuclear membrane; at the same time the kinetonucleus is considerably diminished in size, the periplast-line begins to stand out, and the flagellum is thinner. In the next stage (fig. 65), the trophonucleus is pale with a few scattered coarse granulations, amongst which it is still difficult to identify the karyosome. In the next two figures (figs. 66, 67), it is seen that the coarse granulations are disappearing, leaving the karyosome standing out plainly, with a more or less distinct clearer space round it. In the last two figures (figs. 68, 69), which represent the last stage of extraction and the condition that is most frequently met with in the preparation, the trophonucleus is reduced to an oval space of faint pink colour, in which the karyosome or karyosomes stand out sharp and clear—exactly the same state of things which is found in preparation *c*

(figs. 10, 11), stained with iron-hæmatoxylin, and which results also from other stains, such as methyl-green, Twort's stain, etc. The resemblance between preparation *a*, when fully extracted, and preparation *c*, extends also to other points. The flagellum is fine and delicate in structure, the blepharoplast minute and scarcely visible, and the periplast-line stands out sharply on the concave sides of the body. But there is one remarkable point which puzzled me greatly, and which I took great pains to assure myself was real and not due to errors in representation, namely, that in preparation *a* (figs. 64-69), the trypanosomes are constantly smaller as a whole than in preparation *b* (figs. 62, 63). This is difficult to explain. It may be that the preparation *b* has dried slightly during the process of fixation, and so resisted the tendency to shrinkage, which I believe must always be reckoned with in preparations mounted without drying in Canada-balsam. But this is evidently not the whole of the explanation, for the fully extracted trypanosomes in preparation *a* (figs. 68, 69) are smaller than those in *c* (figs. 10, 11), stained with iron-hæmatoxylin. It seems to me quite possible that when a considerable quantity of stain deposited in the body of the trypanosome is dissolved out, the body may shrink to some extent as the substance of the stain is removed. I do not know how else to explain the distinctly smaller size of the trypanosomes in question.

There is one further point relating to the nucleus which I find very difficult to explain, namely, the frequent appearance, in trypanosomes stained with Giemsa's stain in the ordinary way, of a lighter spot at or near the centre (figs. 61, 70, 80, 81, etc.). From a comparison with preparations in which the stain is more or less extracted, this clear spot appears to correspond to a space or chromatin-free area close beside the slightly excentric karyosome; it does not appear, however, to be always present.

I pass to the consideration of the action of the Romanowsky stain on the flagellum and periplast. It is well known that both these structures stain red with the stain. Schaudinn

first, and after him Prowazek and others, have attributed this to a similarity in origin and substance between the locomotor apparatus and the nucleus. According to Schaudinn (1904, p. 395) the entire locomotor apparatus (*Trypanosoma noctuæ*) is a nuclear product, and the periplast stains red like the nucleus, because it contains myonemes originating from the mantle-fibres of a nuclear spindle. Since Schaudinn wrote, the staining properties of the flagellum have generally been considered as due to its affinities with the chromatin of the nucleus. This is a good instance of the danger of founding conclusions of this sort on the results obtained by a single staining method; for when trypanosomes are stained with Twort's combination of neutral red and Licht-grün, the flagellum, blepharoplast and periplast stain green, in sharpest contrast to the two nuclei, which are both stained red (Pl. 23); hence this method leads to conclusions diametrically opposite to those that can be drawn from the results given by the Romanowsky combination.

I cannot claim to have an expert knowledge of the chemical changes that go on, and the staining substances that result from them in the Romanowsky mixture; but certain conclusions seem to me legitimate, and, indeed, quite obvious. In the first place the mixture contains eosin, or its equivalent, erythrosin. Eosin is not, however, a stain for chromatin, and it cannot be held accountable for the red coloration of the chromatin. When trypanosomes are stained with methylene blue and eosin by Chenzinsky's method, the chromatin stains a pure blue colour. It is generally acknowledged that there is, in the Romanowsky combination, a red stain other than eosin at work which is accountable for the red stain of the chromatin. If it be true, however, that in the Romanowsky mixture at least two red stains are present, the one a stain for chromatin, the other not, it is evident that the fact of two structures being stained red by the mixture is no proof whatever of any similarity in nature or substance between them. Hence the red stain of the flagellum and periplast is not proof in itself of their relationship to chromatin.

(2) Heidenhain's iron-hæmatoxylin.—For this stain I make use of $3\frac{1}{2}$ per cent. solution of iron-alum in distilled water and $\frac{1}{2}$ per cent. solution of hæmatoxylin, which is made up as follows: A stock solution is made in the proportions of 1 gramme hæmatoxylin, 10 c.c. absolute alcohol, 90 c.c. distilled water; for use the stock solution is mixed with an equal volume of distilled water.

In agreement with Schaudinn (1902, p. 190), I found it necessary to let the mordant and stain act for a long time. My procedure is as follows: Films, however fixed, are brought into absolute alcohol and kept there for an hour or so, then brought down through a series of grades of alcohol, differing by 10 per cent. between two consecutive grades, into distilled water, thence into the iron-alum solution, in which they are left till the next morning. The films are then dipped for an instant into distilled water, transferred to the hæmatoxylin solution, and left for at least twenty-four hours. I use both the iron-alum and hæmatoxylin solution in the solid watch-glasses mentioned above, taking care that none of the solution, in either case, gets on to the clean upper surface of the coverslip.

The important part of the whole method is the process of extraction of the stain. After being in the hæmatoxylin the film is washed with distilled water and placed in the iron-alum solution; in a short time clouds of colour can be seen coming out, especially if the coverslip be gently moved; it is then taken from the iron-alum and placed in tap-water, which stops the process of extraction. I now examine the film, in tap-water in a watch-glass, with a dry lens (Zeiss D with oc. 4). If the karyosomes of the trypanosomes can be seen clearly the extraction is sufficient, if not the film is put back again into the iron-alum for a short time, and the process of extraction and examination repeated. When the extraction is judged to be sufficient, the films are washed for twenty minutes in a current of tap-water, which is easily done by letting them float on the surface of the water in a beaker through which a gentle current of water is passed from a

pipe immersed below the surface of the water; the cover-slip will neither sink nor run over the edge of the beaker with the current, and can be washed with safety for any length of time. After the tap-water I put them for a few minutes in distilled water, then pass them up through the grades of alcohol into absolute, in which I leave them for at least an hour, then pass them through xylol into Canada-balsam.

As a rule I have always done several films at the same time, and extract the stain to different degrees in different films (compare figs. 25-28, 29-32). When the trypanosomes come out of the hæmatoxylin they appear uniformly black all through. The stain first comes out of the general cytoplasm; next out of the cytoplasmic granules and the body of the nucleus, leaving the karyosome sharp; with more extraction it is taken out of the flagellum and blepharoplast; the bodies that retain it the longest are the kinetonucleus and karyosome, especially the former. In fish-trypanosomes I was able, in some instances, to see myonemes after moderate extraction when the stain was only removed from the general cytoplasm; but in *Trypanosoma lewisi* I have never succeeded in seeing myonemes.

Iron-hæmatoxylin is the stain of choice for minute structural details of the nuclei and locomotor apparatus. Perfect reliance can, in my opinion, be placed on the results yielded by it, and the stain can always be trusted to give uniform results with the same degree of extraction. It stains most sharply and clearly after sublimate mixtures, less so after osmic vapour and alcohol simply. I have never had good results with it in films dried at any time, before or after fixation. I have also never found any advantage, so far as trypanosomes are concerned, in counter-staining with eosin, orange, etc.; all such processes appear to me simply a waste of time.

(3) Twort's stain.—This is a combination of neutral red and Licht-grün invented by Dr. Twort, of the Bacteriological Laboratory, London Hospital Medical College, who has

kindly furnished me with the following directions for making up and using the stain :

“To prepare the stain make up half-saturated watery solutions of neutral red and of Grüber's light green, using distilled water. Place the neutral red solution in a large open vessel, and add to it sufficient light green solution to combine with the neutral red; the compound will form a precipitate. It is better not to have an excess of either stain, as the precipitate is difficult to wash. When the neutralisation-point is reached, the water will contain a small quantity of both dyes in solution, giving it a dark appearance; the presence of both stains in solution can be easily tested by dropping a drop of the filtrate on to blotting paper; this will spread out, leaving a light red central zone and a faint green outer zone.

“To collect the precipitate it is better not to filter, for the stain soon blocks the pores of the filter-paper. If the solutions are warmed to about 30° or 40° C. before mixing, the precipitate will form in large sticky masses, some of which containing air-bubbles float to the surface and can be removed with a spatula. The rest settle and stick to the bottom and sides of the vessel, and can be collected after pouring off the fluid. The precipitate so collected is rinsed in distilled water and dried at 37° C. In this state it forms dark greenish masses, insoluble in water, somewhat soluble in ethyl alcohol, but soluble to a greater extent in methyl alcohol, and especially so if 5 per cent. of glycerine is added.

“To make up the stain for use, it is best to pound up about 0.25 gm. of the stain with some clean, sharp sand; this prevents the stain going into a sticky mass when the alcohol is added. To the powder so obtained is now added some purest methyl alcohol, acetone-free, containing 5 per cent. by volume of glycerine. Pound up well to obtain a saturated solution; then pour off and add a further quantity of alcohol glycerine solution and repeat the pounding; about 100 c.c. stain can be made from the quantity given.

“The alcohol-glycerine mixture dissolves about .1 per cent.

of the stain, but it is always better to work with an excess of the powder when grinding up, otherwise it is very difficult to obtain a saturated solution.

"The solution when filtered should be kept in a good-stoppered bottle (and if a completely saturated solution has been obtained add 10 per cent. more alcohol-glycerine mixture).

"Stain for five or ten minutes with the stain made up by mixing one part of distilled water with two parts of the glycerine-alcohol stain-solution. Rinse in distilled water. Fix for half to one minute in Unna's glycerine-ether mixture, 2 per cent. in distilled water. Rinse in distilled water.

"Differentiate and dehydrate in absolute alcohol. Should there be much precipitate this can easily be removed by a few drops of methyl alcohol or equal parts of absolute alcohol and xylol. Remove absolute alcohol with xylol and mount in Canada-balsam in the usual way."

I made a number of smears of blood containing *T. lewisi* in order to try the effects of Twort's stain, and also used the stain mixed in different proportions with distilled water, and allowed to act for varying lengths of time. I could not obtain good results with any smears fixed with osmic acid, either when smears previously exposed to osmic vapour were subsequently treated with absolute alcohol or sublimate-acetic or when smears were fixed direct in Flemming's or Hermann's fluids. On the other hand I got excellent results with smears fixed direct in sublimate mixtures, either sublimate-acetic, Schaudinn's fluid, or Mann's picro-corrosive, with or without formol (Pl. 23). As regards the application of the stain I did not obtain good results with weak mixtures used for a long time, but I got my best results by mixing the stain in equal quantity with distilled water, or by using two or even three parts of stain to one of distilled water and allowing the stain to act for from twenty minutes to an hour. I differentiated with 5 per cent. Unna's glycerine-ether solution. I found that when the staining mixture was placed on the slide or coverslip a very dense precipitate formed on the smear, but by placing the stain in a suitable vessel such as a watch-glass,

and putting the slide or coverslip in it with the smear downwards, no precipitate was formed on the smear.

My recommendations for the use of this stain for trypanosomes are, therefore: fix with sublimate mixtures; use the stain strong, 50–75 per cent.; stain for an hour, more or less; and place the smear downwards in the stain.

Twort's stain, for "show" preparations, has the fault that it stains rather faintly; this might, perhaps, be overcome by letting it act longer. On the other hand it gives results which are extremely instructive and important (Pl. 23). The trypanosomes are stained in two colours, red and green. The two nuclei and the chromatoid granules are red; the flagellum, blepharoplast, and periplast are green. The general protoplasm appears to have a greenish tint, perhaps due to the periplast. The structure of the trophonucleus and the size of the kinetonucleus are just as they are after iron-haematoxylin.

Other stains.—I made trial of a number of other stains, or combinations of stains, none of which gave results which could permit me to recommend their use for "show" preparations, but in some cases the results are interesting as reactions.

Delafield's haematoxylin.—Stock-solution made up as recommended in Bolles Lee's 'Vade-Mecum' (5th edition, p. 184). For staining, a few drops added to about 30 c.c. distilled water, acidulated with one drop of glacial acetic acid; films stained in it for several hours, then washed with distilled water, brought up through the grades of alcohol into 90 per cent.; left in this overnight, then mounted in the usual way.

Effects (after Hermann's fluid) very poor; the trophonucleus a narrow dark patch with a clear space round (effect of fixative, see above); kinetonucleus indistinct; flagellum faintly stained; cystoplasmic granules rather deeply stained, giving the body a blotchy, marbled appearance.

I think it highly probable that Delafield's haematoxylin would have given better results after other fixatives. I have recently obtained excellent results with it, on other material,

after fixation with Schaudinn's fluid and Mann's fluid; but as the preparations stained with it are so inferior to those obtained with iron-hæmatoxylin, it did not seem to me worth while to waste time on experimenting with it.

Gentian violet and safranin.—I used these two stains made up as follows: 1 grm. of the stain in 90 c.c. aniline water with 10 c.c. absolute alcohol (Hermann's formula).

Films fixed in Hermann's fluid or Schaudinn's fluid, and stained in gentian violet for half an hour or so, were washed with absolute alcohol, then extracted in absolute alcohol saturated with orange G, then mounted in Canada-balsam after passage through xylol.

Result (fig. 40): Trophonucleus a dark patch with karyosome not distinct; kinetonucleus indistinct; flagellum faintly stained; cytoplasm showing the very same appearance as after Delafield's hæmatoxylin.

Other films, fixed as before, were stained in safranin at least twenty-four hours, washed with absolute alcohol, acidulated alcohol, and absolute alcohol again in rapid succession, then stained in gentian violet and differentiated with orange as already described.

The only effect (figs. 41, 42) produced by the additional treatment with the safranin was to deepen the staining of the cytoplasmic granules, which assume a brownish, muddy tint, and so increase the marbled, blotchy appearance of the cytoplasm.

Methylene blue and eosin.—Used according to Chenzinsky's method, as given in Bolles Lee's 'Vade-mecum' (5th edition, p. 222); films fixed with Hermann's and Schaudinn's fluids. Grübler's methylene blue BX was used.

Result: Exceedingly poor as show preparations, but interesting as showing the two nuclei and the cytoplasmic granules stained blue; no other parts of the body are stained at all, hence the trypanosomes appear shadowy and ghost-like, recognisable at first only by their nuclei and granules, but after careful examination the other parts of the body can be made out. The trophonucleus appears as a blue patch, with

the karyosome not very distinct; the kinetonucleus is sharp and deeply stained, its size the same as after iron-hæmatoxylin. The body shows blotchy blue granules, varying in amount in different specimens.

Carmine stains.—I have tried picrocarmine and other carmine stains on trypanosomes, but have never obtained any results worth mentioning.

One cause of the defective results given by some of the stains mentioned above—for instance, Delafield's hæmatoxylin, safranin, gentian violet—seems to me to be in the fact the stain colours the cytoplasmic granules so deeply as to obscure other parts, especially the trophonucleus, which stains less deeply.

It must, I think, be the experience of every one who has tried different methods of technique on different objects, as it certainly has been my experience, how impossible it is to infer or predict the results that a given method will yield for a particular object from the results that it yields on other objects. This truth is constantly brought home to anyone studying trypanosomes, as one so frequently obtains preparations in which the trypanosomes are vile, while the leucocytes are beautifully stained. Thus in films stained with Twort's stain after Hermann's fluid, the leucocytes leave nothing to be desired, while the trypanosomes are useless. Similarly in my preparations stained with methylene-blue-eosin the leucocytes are exquisite.

I have had some experience of the results of technique on another class of objects which might be expected to be not so different from trypanosomes in their reactions, namely, the collar-cells of sponges. A collar-cell is a flagellate organism which might be described as possessing a non-undulating membrane, perhaps similar, morphologically, to that of trypanosomes, but not connected with the flagellum and not contractile in the same manner, only slowly retractile. No *a priori* reason presents itself to me that would lead me to expect a trypanosome and a collar-cell to be very different in their staining reactions.

Beautiful preparations of collar-cells can be obtained by fixation in osmic acid followed at once by staining with picrocarmine; the same method is absolutely useless for trypanosomes. The osmic-picrocarmine method shows the nucleus of the collar-cells as a deeply stained pink mass, in which a nucleolus can just be made out, but beautiful preparations of nuclear structure in collar-cells can be obtained by fixation in Flemming's or Hermann's fluids followed by Delafield's hæmatoxylin, safranin, gentian violet, methylene-blue-eosin, etc.—just the methods, in fact, which I used on trypanosomes with such ill success (but with excellent results on the leucocytes).

It seems to me, therefore, quite illogical to argue that a method is good or bad for trypanosomes or any other cells, because it is good or bad for some quite distinct class of object. Every kind of cell requires its own special technique, which must be established empirically by trial, and can be discovered only to very limited extent and with great uncertainty by analogy.

PART II.—THE STRUCTURE OF *TRYPANOSOMA LEWISI*.

(1) General Structure, Form and Dimensions.

As I have stated above, I believe that the preparations fixed simply with osmic acid vapour, and examined in the blood with or without addition of acidulated methyl-green but without further treatment of any kind, represent the nearest possible approach to the living condition in form and dimensions. In such "standard" preparations (figs. 1-8) the body of the trypanosome appears long and slender, sharply marked off from the distinct undulating membrane and flagellum. The kinetonucleus is seen near one extremity, which for convenience may be termed "posterior." The trophonucleus is seen as an oval, well-defined space, containing the distinct karyosome, and situated at rather more than two thirds the length of the body from the posterior end. We

may, therefore, for purposes of description, divide the body into three regions: the pre-nuclear region in front of the trophonucleus; the inter-nuclear region between the two nuclei; and the post-nuclear region behind the kinetonucleus.

In the inter-nuclear region the body is roughly cylindrical, but slightly thicker midway between the nuclei. In the pre-nuclear region the body tapers very rapidly to a fine filamentous prolongation, often difficult to distinguish from the flagellum. In the post-nuclear region the body also tapers evenly and rapidly to a point.

The flagellum arises from the blepharoplast or basal granule situated in front of the kinetonucleus, and runs at once slantingly forwards up to the surface of the body to form the marginal flagellum, running along the edge of the undulating membrane and continued beyond the anterior extremity as the free flagellum. The breadth of the undulating membrane appears greater or less, according to the view presented by the trypanosome; so far as can be judged, it is rather more than half the greatest thickness of the body. In this trypanosome the undulating membrane is not much pleated and runs in even, sinuous curves, corresponding to the twists of the body. In any preparation the trypanosomes are found in all sorts of positions, but, speaking generally, the body usually shows one or two main curves, and may be briefly described as either more or less **C**-shaped or **S**-shaped. In either case the undulating membrane keeps always to the convexity of the curves, crossing over the body between each bend. The explanation of this is to be found in the fact that the marginal flagellum is considerably longer than that portion of the body along which it runs, as can be determined easily by actual measurement; it is shown in the table of measurements given below that the average length of the pre-nuclear and inter-nuclear regions together is $19.26\ \mu$, while the average length of the marginal flagellum is $23.51\ \mu$. If we imagine the marginal flagellum as an elastic fibril joined by the undulating membrane to a considerably

shorter body, plastic and (after death) inert, we can understand the body being thrown into curves by the elasticity of the flagellum, which, being longer, necessarily runs on the outside of the curves. Only in a single instance have I seen the marginal flagellum on the concave side of a curve in a preparation of *T. lewisi* (fig. 9), and in this case the flagellum showed a double bend with sharp angles at this point, suggesting a forcible pleating of the flagellum, owing to the fact that the body, wedged in between blood-corpuscles, was unable to take the curvature which the elasticity of the flagellum would naturally cause.

I give in a table below a number of measurements of ten trypanosomes from standard preparations made in the following way. The trypanosomes were first of all carefully drawn with the camera lucida, projected to a magnification of 3000 linear, as determined by substituting for the trypanosomes a stage-micrometer, of which the divisions were drawn in the same manner and the resulting scale measured. The magnification thus obtained depends, other things being constant, on the height of the camera lucida from the drawing board, that is to say, on the length of the tube of the microscope. By trial a length of tube was found, which, with the combination of lenses used, projected the divisions of the micrometer-scale, each representing $\cdot 01$ mm., to a scale in which they were 30 mm. apart. Taking the magnification of 3000 obtained in this way as accurate, the measurements shown in the table were obtained.

The measurements were carried out by following the undulations of the trypanosomes in the figures with a piece of thread, the length of which was then measured in millimetres; the figure obtained was divided by three, and the resulting figure represents, therefore, the actual length in microns. The pre-nuclear region is measured from the karyosome, or when two nuclei are present (fig. 6) from the boundary between them, to the extreme anterior end of the body; the free flagellum from the latter point to the end. As it is often difficult to determine exactly the anterior

termination of the body, these two measurements are a little uncertain and subject to considerable variation. The inter-nuclear region is measured from the karyosome to the centre of the kinetonucleus; the post-nuclear length is from the kinetonucleus to the posterior extremity of the body. The undulating membrane is measured by following up the sinuosities of the marginal flagellum from the kinetonucleus to the anterior extremity of the body.

Total length (body and free flagellum).	Body.				Flagellum.	
	Pre- nuclear region.	Inter- nuclear region.	Post- nuclear region.	Greatest breadth.	Undulat- ing mem- brane.	Free flagellum.
(Fig. 1) 28	—	—	—	1.5	21	7
(Fig. 2) 32	—	—	—	2	24	8
(Fig. 3) 30.5	6	12	4.5	2	21	8
(Fig. 4) 28.6	6	11	4	1.6	27.6	7.6
(Fig. 5) 28	8	11.3	3.3	1.6	21.4	5.6
(Fig. 6) 31	7	12.5	5	1.6	24	7
(Fig. 7) 33	6.3	13	5.3	1.5	25	8.3
(Fig. 8) 32	9	12.5	5	1.5	24.6	5.3
32.3	8	12.5	5	1.5	24	7
30.3	6	13	4	1.6	22.5	7
Average 30.57	7.04	12.22	4.5	1.64	23.51	6.88

Salvin-Moore, Breinl, and Hindle have recently (1908) published an elaborate study of *Trypanosoma lewisi*. Since they deal chiefly with the multiplicative period, when the trypanosomes vary enormously in size and form, it is difficult to compare their results with mine. Their fig. 1 appears to represent a so-called "adult" trypanosome, in the period when multiplication is past, comparable to the forms with which I am dealing in this memoir. If that is so, I think anyone who has the most elementary acquaintance with *T. lewisi* will agree with me that their technique has distorted and deformed the creature very greatly. This is not surprising to me, since these authors rely on Flemming's fluid as

a fixative, and I have always found this mixture to give the worst results of any that I have tried as regards the general form of the body (compare my figs. 36-38).

It does not, however, follow that because the external form is distorted the internal structure is necessarily falsified. I have got the sharpest differentiation and clearest pictures of the cytological details in preparations fixed with sublimate mixtures, in which the body as a whole is undoubtedly diminished in size. But I do not think that the technique of Salvin-Moore and Breinl (1907) gives them any right to deny the very obvious trimorphism seen in *T. gambiense*, for instance, since their preparations, to judge by their illustrations, are obviously inferior to the ordinary osmic-vapour Romanowsky preparations for a study of the form, size, and general structure of the body. I have elsewhere contested their statement that *T. gambiense* shows no form-differentiation. *T. lewisi*, on the other hand, is certainly remarkably uniform in structure during the non-multiplicative period. It is not possible to distinguish any distinct types of form, nor anything but slight variations in size, as shown in my table above. The differences that can be observed in the kinetoneuclei of different forms are dealt with below.

(2) The Periplast.

That trypanosomes have a fairly strong and resistant membrane or cuticle at the surface of the body is obvious from the manner in which they preserve their body-form under trying circumstances. This fact is brought home to anyone who has studied the trypanosomes and trypanoplasms of fishes. As I have shown elsewhere,¹ in smears of fish-blood dried before fixation the trypanosomes may be almost perfect in form and appearance, while the trypanoplasms side by side with them in the same preparations are deformed almost beyond recognition. This alone is a sure indication that the body-cuticle, commonly termed periplast, is very delicate in the trypanoplasms but comparatively strong in the trypanosomes.

¹ 'Proc. Zool. Soc.,' 1909, pp. 3-31, Pls. I-V.

In *Trypanosoma lewisi* it is not difficult to discover the existence of a fairly thick periplast, which can be stained and shown up by a variety of methods. After the Romanowsky stain it is usually seen at the edge of the body as a red line, most distinct as a rule on the side opposite to the flagellum, where sometimes it is almost as deeply stained as the flagellum itself (figs. 80, 81). With Twort's stain the periplast stains green and is often very distinct (Pl. 23). With iron-hæmatoxylin the periplast stains very faintly and appears excessively delicate.

In many preparations the periplast becomes very distinct, not by being coloured itself, but by the layer of the body immediately under it becoming clear and transparent, so that the periplast appears, on the side opposite to the flagellum, to stand out from the body as a delicate line, very easily overlooked in preparations in which it is not stained. Figs. 64-69 show very well the genesis of this periplast-line in the process of extraction of Giemsa's stain. It is also seen well in the preparations fixed with Schaudinn's fluid followed by Twort's stain (fig. 85), which colours the periplast green, as already stated.

In preparations stained with Romanowsky stain, after being preserved in various ways, and either dried off or mounted in Canada-balsam without ever being dried, some trypanosomes may be found showing creases and folds of the periplast, which are stained red and simulate fibrils running more or less longitudinally (see especially figs. 70-72). I shall return to this point again later on.

In or immediately under the periplast there are found in many species of trypanosomes, as is well known, distinct contractile fibrils or myonemes. I have myself seen very clearly, and described elsewhere, myonemes in *Trypanosoma percae*; less distinctly in *Trypanosoma granulosum*. I found them most clearly shown in preparations fixed first with osmic vapour, then with Schaudinn's fluid, and stained with iron-hæmatoxylin, very slightly extracted.

In *T. lewisi* I have not succeeded in seeing myonemes, in spite of much searching of preparations fixed and stained in

various ways. I do not know whether my failure to find them is due to the minuteness of the object, or to my not having hit off just the right degree of extraction of the iron-hæmatoxylin stain. The trypanosomes in which I have seen myonemes have been very large species, and it is possible that in *T. lewisi* the myonemes are too minute to be resolved with the magnification used. On the other hand, myonemes appear to give up the stain very readily, while if over-stained they are obscured by the darkness of the preparation. There can be hardly any doubt that these active flexible organisms must possess a contractile apparatus which suitable methods of technique or optics would reveal.

(3) The Cytoplasm.

In the thickest part of the body, that is to say, in the inter-nuclear region and immediately in front of the trophonucleus, the cytoplasm shows commonly in preparations two distinct regions, a peripheral zone, situated immediately below the periplast, and an axial portion.

The peripheral zone is usually seen in the ordinary Romanowsky preparations as a more or less distinct border marked off by its red tint from the axial bluish region (figs. 60, 61). When the stain is extracted, however, the peripheral zone becomes clear and apparently empty, leaving the periplast-line standing out in the manner already described (figs. 62-69). It is quite evident, from a comparison of different specimens, especially as regards breadth, that the appearance of the periplast-line is really due to the clearing up of the region immediately below the periplast and not to a dilatation of the periplast, or artificial raising up of it from the body. The question is, How is the clearing up of the region under the periplast effected? Is it a clear zone of protoplasm which takes the red stain, and from which the stain is extracted, or is it really an empty space in which the stain is deposited and from which it is dissolved out again? And if it is an empty space is it one naturally present, filled only with fluid in the living condition, or is it produced artificially by shrink-

age and contraction of the body-cytoplasm? These are difficult questions to answer, but I think that, so far as the trypanosomes in the preparations are concerned, the clear zone is shown to be really a space: first, by a comparison of the trypanosomes fixed by certain methods, for example, Mann's fluid (figs. 29-35, 77-79, 86, 87), in which a comparison with other preparations shows clearly that the thinness of the body is due to an obliteration of the clear area under the periplast by shrinkage, so that the periplast comes into contact with the axial portion of the cytoplasm; secondly, from the frequent creases and folds in the periplast already mentioned, indicating the existence of a space under it which is either empty or at least not completely filled out.

Since, on the other hand, no trace of a clearer peripheral zone can be seen in the living trypanosome nor in the standard preparations, I am inclined to think that the appearance of the peripheral clear zone under the periplast is due simply to shrinkage of the cytoplasm in preparations, leaving a space at the periphery in which the red stain of the Romanowsky combination becomes deposited. This conclusion may be supported also on general grounds; it is common in protozoa to find a more fluid endoplasm surrounded by a less fluid ectoplasm. It is, on the other hand, very unusual to find the peripheral region of the cytoplasm of more fluid nature than the region situated more internally.

For all these reasons I am of opinion that the clear space seen under the periplast is an artefact, the result of shrinkage of the cytoplasm produced by the processes of dehydration and clearing, necessary when never-dried preparations are mounted in Canada-balsam. No such space is ever found in dried-off preparations.

Apart from the nuclear and locomotor apparatus, the cytoplasm contains various enclosures. First in importance are certain granules situated chiefly in the inter-nuclear region; as it is convenient to employ a distinctive term for them, I propose to use the term chromatoid grains.¹

¹ Compare Woodcock, 'Quart. Journ. Micros. Sci.,' vol. 50, p. 229.

These bodies are best seen after Twort's stain, which colours them red in the midst of the greenish cytoplasm (figs. 82-87). They are also well seen after iron-haematoxylin if the stain be but little extracted (figs. 28-30), and are most distinct when the trophonucleus appears as an evenly stained black patch without any detail; if, however, the extraction be carried further the stain comes out of them, and in preparations in which the karyosome stands out sharply from the trophonucleus the chromatoid grains are no longer visible (figs. 14, 15, 25, 31, 32). With the Romanowsky stain the chromatoid grains can seldom be made out; sometimes (figs. 61, 74, 80) a few red granules are to be made out, but more usually the cytoplasm stains an even bluish or purplish tint, which may mean either that the chromatoid grains are stained red, but are obscured by the dense blue colour of the general cytoplasm, or that they also take the blue coloration of the Romanowsky stain. After Delafield's haematoxylin the chromatoid grains stain a dull violet like the nucleus; after gentian-violet-orange and safranin-gentian-orange they also stain like the nucleus; after methylene-blue-eosin they take the blue colour. With all these stains, however, and also with iron-haematoxylin, the general cytoplasm is tinged in the same manner, probably owing to the presence of minute granulations diffused through it which also take up the stain. Hence the chromatoid grains do not appear after the five staining methods mentioned as definite sharply marked bodies, but as blotchy, ill-defined patches which give the cytoplasm a marbled appearance.

Twort's stain alone, of all I have tried, differentiates the chromatoid grains clearly from the surrounding protoplasm. By this method they appear as coarse granules stained a faint reddish tinge, irregular in form and not sharply contoured. They vary in amount, being sometimes spread over the whole inter-nuclear region, and even extending into the pre-nuclear region; in other cases there are only a few of them, occurring chiefly just behind the trophonucleus (figs. 82-87). These variations in the quantity of the chromatoid grains do not

appear to be correlated with any other structural variations of the trypanosome.

By their reactions to the stains mentioned, the chromatoid grains are evidently allied to the chromatin-elements of the nuclei; they are stained in a manner similar to the trophonucleus by hæmatoxylin, gentian-violet, safranin, methylene-blue, and neutral red (in Twort's stain). The Romanowsky stain alone fails to differentiate them clearly, but I have said enough already in support of my opinion that this stain is quite unreliable as a test for nuclear structures. Comparing the chromatoid grains with the constituents of the trophonucleus (see below), it is clear that their reactions agree very closely with the intra-nuclear chromatin. In preparations in which the stain is extracted from the intra-nuclear chromatin, leaving the karyosome sharp, it is also extracted from the chromatoid grains. When the intra-nuclear chromatin is coloured, the chromatoid grains are also coloured, and to about the same tint. On these grounds I infer that the chromatoid grains represent extra-nuclear chromatin or chromidia, derived from the trophonucleus, possibly from the karyosome (see below).

In ordinary preparations the cytoplasm does not contain any other enclosures than the chromatoid grains. In my standard preparations, however, examined in the wet blood, I find a body which is not to be made out in trypanosomes examined in Canada-balsam or cedar oil (figs. 1-8). Quite constantly a refringent granule is seen, sharply and distinctly, in the post-nuclear region, sometimes close behind the kinetonucleus, sometimes nearer the pointed posterior termination of the body. It has the usual appearance of a refringent granule appearing as a black dot at the lower focus and as a clear spot at the higher focus. It is more refringent and much more distinct than the unstained kinetonucleus. At first I thought it might be Prowazek's "anchoring granule," but I do not find anything anchored to it, and it disappears in the stained and mounted preparation. This is probably due to the fact that the granule itself does

not stain, and is only visible by its refringence, and consequently disappears when mounted in refractive media. As to the nature or function of the granule, I am not able to offer any suggestions.

In many trypanosomes in the standard preparations I find a distinct clear space or vacuole immediately in front of the kinetonucleus, varying in size, frequently absent, sometimes double (figs. 5-8). This is perhaps an artefact or post-mortem change, as I have not been able to see it in the living animal, nor does it occur in the ordinary stained preparation.

(4) Locomotor Apparatus.

Under this head are comprised the flagellum with its basal granule or blepharoplast, and the undulating membrane. I have already described above the general configuration of the flagellum and undulating membrane, and shall deal here only with points of minute structure.

The blepharoplast is situated very close to the kinetonucleus, apparently touching it or even overlapping it; that is at least the position which it has in the standard preparations (figs. 1-8) and in all preparations which have been mounted without ever having been dried (compare figs. 62-69). On the other hand, in preparations which have been dried off, either before or after fixation, a considerable interval usually separates the kinetonucleus and blepharoplast (figs. 60, 61, 70, 73, 80, 81), a state of things which must be regarded as artificial. In view of the alleged purity of the technique of Salvin-Moore, Breinl, and Hindle (1908), I am surprised to notice such a condition very frequently in their figures.

In preparations stained with iron-haematoxylin or other sharp nuclear stains the blepharoplast appears as a minute granule, a slight dilatation, often hardly visible, of the proximal end of the flagellum (figs. 25-32, etc.). After Twort's stain it is coloured green, like the flagellum itself, in sharp contrast with the nuclei, which are red (figs. 82-87). After the Romanowsky stain the blepharoplast is coloured red, like

the flagellum and the nuclei, and instead of appearing as a very minute dot, it is usually considerably enlarged in size, appearing sometimes as a relatively large, diffuse patch (figs. 70, 73). I regard this appearance of the blepharoplast simply as another instance of the tendency of the Romanowsky stain to form concretions, as it were, round the bodies for which has the affinity, whatever it may be, which is shown by staining.

The flagellum presents itself as a delicate filament of even thickness and like appearance throughout its whole length (figs. 10, 11, etc). I have not been able to see any structural differences in it in different parts. After the Romanowsky stain the flagellum commonly appears much thicker and quite coarse in structure, a state of things evidently due to the usual performances of this staining method. When, however, the Romanowsky stain is suitably extracted, the flagellum comes down to its normal thickness (figs. 67-69).

Prowazek (1905) has introduced a most extraordinary complication of fibrils into the structure of *T. lewisi* (see his text-fig. 2B, p. 359). One fibril is supposed to connect the karyosome with the kinetonucleus; from the latter another fibril runs to an "anchoring grain" situated in the post-nuclear region, and from this grain yet another fibril runs forward to the anterior end of the body. I have searched in vain in all my preparations, without finding a trace of this fibrillar system. In my smears stained with the Romanowsky stain, however, I find that the periplast, stained red, shows creases and folds often closely simulating distinct fibrils. I have represented some of these in figs. 70-72 (Pl. 22), and could have drawn many more. I have a strong suspicion that Prowazek's fibrillar system is founded on nothing but accidental creases of this kind, since he remarks, "Die Fasern selbst lassen sich färberisch nur schwer darstellen und nur an weniger Objekten konnte man sie streckenweise als rote Fibrillen verfolgen" (loc. cit., p. 358). This so exactly describes what I have seen that I must express my profound scepticism as to the existence of Prowazek's fibrillar system.

(5) The Nuclear Apparatus.

Under this head I include simply the two nuclei, trophonucleus and kinetonucleus. I will begin my account with the second of these two bodies.

The kinetonucleus is easily made out in the living condition or in the standard preparations as a refringent body of fair size a short way from the hinder end. It is also seen, especially in the fixed preparations, that there are two types of form presented by the kinetonucleus, which may be either rod-shaped or circular in contour. Transitions occur, but not commonly, between these two types.

With iron-hæmatoxylin the kinetonucleus stains black; the rod-like type stains much more darkly than the round form, which appears of a pale greyish tint when the stain is well extracted; it is then not so dark in tint as the blepharoplast, which is seen as a black dot on the edge of the kinetonucleus (figs. 31, 33). The rod-shaped type of kinetonucleus, on the other hand, takes the stain more darkly than the blepharoplast which is seen close beside it, not overlapping it (figs. 32, 34).

By all nuclear stains the kinetonucleus is stained intensely, more so than any other part of the body, in fact it may be the only part of the body visible in the preparation. After methylene-blue-eosin it is blue; after Twort's stain, red; after the Romanowsky stain, as is well known, it stains a deep purple-red; what is not so generally known is that it comes out, as I have already pointed out, four or five times its natural size (compare figs. 62, 63, with figs. 10, 11). When, however, the Romanowsky stain is suitably extracted, the kinetonucleus comes down to the size that it appears in other preparations (figs. 66, 69, etc.).

I have not been able to make out any structure in the kinetonucleus. It appears to consist simply of a dense mass of chromatin, which is carried on, or impregnates, a basal substance, for which the name "plastin" is in common use.

With regard to the two forms of the kinetonucleus, it is possible that these may be simply two views of the same

thing, a body having the form of a disc which, seen edgeways, would appear rod-shaped, and in surface view would present a circular contour. This explanation is compatible with the facts stated above, namely that the rod-shaped type is darker in colour, that there are transitions between the two forms, and that the blepharoplast overlaps the round form, but is found close beside the rod-shaped form. I have not been able to convince myself that this explanation is either true or false.

Salvin-Moore, Breinl, and Hindle (1908) are of opinion that the kinetonucleus is to be regarded as of the nature of a centrosome or blepharoplast, and use the term "end-bead of the flagellum" for the granule termed by me the blepharoplast. The question is one that must be decided by developmental data; a true centrosome is pre-eminently a body of dynamic rather than static function. I do not desire, therefore, to argue the question in this memoir, in which I purposely avoid dealing with division-stages. I will only say that in refusing to allow the kinetonucleus the status of a true nucleus, the authors are ignoring a great deal of work by Schaudinn and others in support of this view. As regards the "end-bead," I am still of opinion that it represents a true centrosome or blepharoplast. In *Trypanosoma grayi* I found that division of the blepharoplast was invariably the first step in the division of the trypanosomes.

The trophonucleus is at first very puzzling, on account of the extraordinary difference in its appearance after the Romanowsky stain, now so much in use, and all other stains. The coarse "Romanowsky splotch" seems at first sight to have nothing in common with the delicate, refined structure seen after iron-hæmatoxylin or Twort's stain (compare figs. 60-63, with figs 10, 11, and 82-87). I have already attempted above an explanation of the vagaries of the Romanowsky stain; my reasons for regarding the results of this stain as departures from the truth are simply founded on the facts—first, that the condition seen after iron-hæmatoxylin is also seen in the standard preparations, in the living condition, and after every other nuclear stain known to be of value as such;

secondly, that when the Romanowsky stain is cautiously extracted the nucleus comes down to a condition similar to that seen after other stains (figs. 68, 69).

The parts of the trophonucleus may be considered under the heads of the "membrane," the "karyosome" or "karyosomes," and the "intra-nuclear chromatin," the latter term being understood to be exclusive of the karyosome.

The membrane appears as a faint but distinct line circumscribing the oval contour of the nucleus. It does not, however, stand out sharply from the contents, nor does it stain a different colour from the rest of the nucleus; it also never shows a double contour. For all these reasons the membrane may be regarded simply as the superficial limit of the intra-nuclear chromatin, condensed to form a more closely knit zone at the surface. In other words, the membrane is not a true nuclear membrane in the sense in which the term is used for Metazoan nuclei.

The karyosome appears usually as a single small grain at or near the centre of the nucleus; this is the commonest condition, found in about 80 to 90 per cent. of the trypanosomes. I regard this, therefore, as the normal state of the nucleus, and will deal with it first, and describe afterwards other conditions. In order to see the karyosome clearly it is necessary to extract the stain thoroughly when staining with iron-hæmatoxylin, otherwise the karyosome is obscured by the granules of the intra-nuclear chromatin. The extraction should be carried on until the karyosome appears as a sharp, clear, and definite body. With Twort's stain no such precautions are necessary, as the intra-nuclear chromatin does not take it up to the same extent, hence this stain is one of the best for showing the karyosome and the nuclear structure generally. In its reactions the karyosome is very similar to the kinetonucleus, but stains less deeply and gives up the stain more readily. I infer from this that the karyosome is, like the kinetonucleus, a body consisting chiefly of chromatin on a basis of plastin, but that the chromatin is much less concentrated and condensed in the karyosome.

The karyosome shows very considerable variations in some of the nuclei. In the first place it varies in size, being usually quite a minute granule, but sometimes very much larger. In the second place it varies in position, and instead of being central or sub-central it may be very excentric in position, even to the extent of being placed quite at one pole of the oval nucleus. Thirdly, the karyosome, usually single, may become double or multiple, apparently through a process of division or disruption. I have described elsewhere¹ such a process of budding on the part of the karyosome in the case of *Trypanosoma granulosum*, where the large size of the object makes the process easy to follow out in detail. The minuteness of *T. lewisi* makes it much more difficult to be quite certain what exactly takes place. Appearances can be found, however, which indicate clearly a process of division or budding from the karyosome (fig. 50), leading to the formation of two karyosomes, subequal or markedly unequal in size; the two bodies thus formed travel apart, and may place themselves at opposite poles of the nucleus (fig. 49). One or both of the karyosomes may undergo further disruption, apparently, so that in addition to a principal karyosome there are smaller granules present, not more than four in number in any case that I have observed (figs. 52, 53, 90); I feel, however, considerable doubt whether some of these smaller granules may not be, in most cases, granules of the intra-nuclear chromatin from which the stain has been insufficiently extracted. In my preparations stained with Twort's stain I find deviations from the condition with a single sub-central karyosome excessively rare; I have, however, seen in these preparations the condition with a double karyosome (fig. 86), and with a larger and two smaller karyosomes (fig. 90).

Prowazek (1905, pp. 361-363) has sought to bring the various conditions of the karyosome under three processes, which he terms "autosynthesis," "reduction," and "parthenogenesis." In autosynthesis the karyosome is supposed

¹ 'Proc. Zool. Soc.,' 1909, pp. 21, 22, pl. V, figs. 78-93.

to divide into two, and each half divides again; two of the four parts are thrown out, the other two parts "copulate," and the nucleus is reconstituted. Equally complicated processes are seen in reduction and parthenogenesis.

For my part I am very doubtful if the different appearances seen in the nuclei of *T. lewisi* will bear the excessive weight of subjective interpretation which Prowazek places upon them. Moreover, Prowazek seems to have based all this theoretical superstructure upon Romanowsky-stained preparations, which, in my opinion, are altogether false and misleading for minute nuclear structure.

All that can be inferred from a comparison of different trypanosomes is that the karyosome sometimes gives off smaller portions; some of these go to the surface of the nucleus, and I think it highly probable that the chromatoid grains described above take origin in this way.

The intra-nuclear chromatin shows different conditions according to the stain used and the degree of extraction of the stain. With iron-hæmatoxylin, for instance, the whole nucleus is at first a dense black patch, owing to the intra-nuclear chromatin being stained so deeply as to obscure completely the karyosome. A similar condition is seen after the Romanowsky stain. As the stain is extracted, the intra-nuclear chromatin gradually becomes lighter in tint, and the karyosome begins to stand out. The extraction of the colour from the intra-nuclear chromatin does not take place uniformly, however, but some of the granules retain the stain longer than others, while some parts become quickly decolorised; there is very often a distinct clear halo round the karyosome (figs. 17, 18, 39). With complete extraction the intra-nuclear substance becomes pale and gives the impression of an empty space, in which the karyosome is suspended (fig. 10, 11). A quite similar and parallel series of appearances is seen when the Romanowsky stain is slowly extracted, as described above, but in this case the granules in the intra-nuclear space are much larger, more of the nature of concretions, than they are after iron-hæmatoxylin. With Twort's

stain the intra-nuclear substance appears a pale red tint, and does not show granulations (Pl. 23); with methylene-blue-eosin it is pale blue. In the standard preparations stained with methyl-green no granulations are to be made out in the nucleus, which has simply the appearance of an oval vesicle, in which the distinct karyosome is suspended (figs. 1-8).

From all these various appearances I conclude that the intra-nuclear space contains, probably, in the living condition a more or less fluid nuclear sap, in which chromatin is suspended in the form of particles, perhaps differing in size or in concentration of substance, but for the most part too minute to be resolved with the microscope, except when very deeply stained, or artificially enlarged by the deposit of the stain round them.

In the foregoing paragraphs I have dealt with the minute structure of the trophonucleus; I will now mention some of its variations as a whole. In the first place it varies considerably in size, as may be seen from my figures. There may sometimes, moreover, be two trophonuclei present, flattened against each other and each of relatively small size (figs. 6, 84). This condition, which is found in a very small number of the trypanosomes, does not appear to have anything whatever to do with division, as all other parts of the body are in the normal single condition; I regard it simply as an abnormality. In rare cases trypanosomes are met with having three trophonuclei (fig. 74).

In preparations fixed with Flemming's fluid (figs. 36-38) and Hermann's fluid (figs. 40-44) the trophonucleus often seems to float in a space in the cytoplasm, a condition which must be regarded as an artefact.

In this memoir I have only dealt with the structure of *Trypanosoma lewisi* in the resting, "adult" stage. It is my hope in a future memoir to extend these studies to the multiplication-period, and possibly to other developmental periods of the life-history.

LISTER INSTITUTE.

January, 1909.

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EXPLANATION OF PLATES 21–23,

Illustrating Mr. E. A. Minchin’s paper on “The Structure of *Trypanosoma lewisi* in Relation to Microscopical Technique.”

All the figures represent preparations of *Trypanosoma lewisi*, drawn with the camera lucida at a magnification of 3000 diameters (see p. 760).

In order to facilitate the comparison of the results obtained by different methods, the figures may be best classified by the dates on which the trypanosomes represented were preserved. All those of a given date were from the same blood, preserved or stained by various methods.

February 24th.—Pl. 21, figs. 14, 15, 19–22, 39; Pl. 22, figs. 60, 61, 70, 73, 80, 81). (The preparations of this date were made on slides; all others, except fig. 74, were made on coverslips.)

March 5th.—Pl. 21, figs. 9–13, 36, 37; Pl. 22, figs. 62–69.

March 16th.—Pl. 21, figs. 23, 24, 40–53.

March 20th.—Pl. 21, figs. 25–34, 54–59; Pl. 22, figs. 76–78.

March 27th.—Pl. 21, figs. 17, 18, 38; Pl. 22, fig. 75.

March 30th.—Pl. 23, figs. 82–84, 88–91.

April 3rd.—Pl. 23, figs. 85–87.

April 6th.—Pl. 21, figs. 1–8, 16, 35; Pl. 22, figs. 71, 72, 79.

The following is a classification of the figures according to the method of fixation employed for the preparations:

Osmic vapour simply: Pl. 21, figs. 1-8.

Osmic vapour followed by absolute alcohol: Pl. 21, figs. 9-11; Pl. 22, figs. 60-69, 70, 71, 72, 74.

Osmic vapour followed by sublimate-acetic (95:5): Pl. 21, figs. 14-18; Pl. 22, figs. 70, 73, 75.

Osmic vapour followed by Schaudinn's fluid: Pl. 21, figs. 23, 24, 45-53.

Osmic vapour followed by Flemming's fluid: Pl. 21, fig. 39; Pl. 22, figs. 80, 81.

Sublimate-acetic (99:1): Pl. 21, figs. 12, 13.

Sublimate-acetic (95:5): Pl. 21, figs. 19-22; Pl. 23, figs. 82-84, 88-91.

Schaudinn's fluid: Pl. 21, figs. 25-28; Pl. 22, fig. 76; Pl. 23, fig. 85.

Mann's fluid without formol: Pl. 21, figs. 29-32; Pl. 23, fig. 86.

Mann's fluid with formol: Pl. 21, figs. 33-35, 54-59; Pl. 22, figs. 77-79; Pl. 23, fig. 87.

Flemming's fluid: Pl. 21, figs. 36-38.

Hermann's fluid: Pl. 21, figs. 40-44.

Figs. 19-22, 39, 60, 61, 70, 73, 74, 80, and 81 are from preparations dried off after fixation or after staining; all others are from never-dried preparations.

In studying the preparations, at least three trypanosomes were drawn from each, in order to take the average of the individual variations, which, though slight, must still be reckoned with, as seen from figs. 1-8 and the table on p. 789. It was, however, neither possible nor desirable to reproduce all these figures. Where only one or two figures are given from a preparation it must be understood that they represent, as far as possible, the average proportions of the trypanosomes in the preparation. Hence such figures as 21, 22, 75, 79, etc., are not to be taken as representing trypanosomes of small size, but as normal individuals showing the shrinkage produced by the method of preparation, and should be compared with others of the same date (see above) prepared in other ways.

PLATE 21.

Figs. 1-8.—“Standard” preparations (see p. 759). 1 and 2 fixed with osmic vapour in a hanging drop; owing to the thickness of the preparation details could not be made out, but only the outline of the body and the flagellum could be drawn. 3 and 4. Fixed with osmic vapour in a hanging drop, then mounted on a slide and sealed up; the trophonucleus with its karyosome, kinetonucleus, and posterior refringent granule are seen. 3 has a rod-shaped, 4 a rounded kinetonucleus.

5-8. Treated as 3 and 4, but with the addition of acidulated methyl-green solution to the preparation. The same details are seen as in the foregoing, with the addition of a vacuole or vacuoles immediately in front of the kinetonucleus, probably to be regarded as artefacts.

With figs. 1-8 compare especially figs. 16, 35, 71, 72, 79, all drawn from preparations made from the same blood at the same time, but preserved in different ways.

Fig. 9.—Outline of a specimen from the same preparation as figs. 62, 63, showing the flagellum passing on the concave side of a body-curve at a point near its origin, and here having a sharp double bend.

Figs. 10, 11.—Osmic vapour, absolute alcohol, iron-hæmatoxylin, never-dried. Note the distinct periplast-line. Compare especially with figs. 12, 13, 36, 37, 62-69, all preserved at the same time and from the same blood.

Figs. 12, 13.—Sublimate acetic (99:1); 12, stained Giemsa; 13, stained iron-hæmatoxylin; never-dried. Compare with figs. 10, 11, etc.

Figs. 14, 15.—Osmic vapour followed by sublimate-acetic (95:5), stained iron-hæmatoxylin; never-dried. Compare figs. 19-22, 39, 60, 61, 70, 73, 80, 81, all preserved at the same time and from the same blood.

Fig. 16.—Fixation as in the last; stained Giemsa; never-dried. Drawn in outline to show the great diminution of size. Compare figs. 1-8, etc., made from the same blood at the same time. All the trypanosomes in the same preparation and in another treated in the same way at the same time show the same reduction in size.

Figs. 17, 18.—Fixation as in 14-16; stained iron-hæmatoxylin; never-dried. Compare with figs. 38 and 75, both preserved at the same time from the same blood.

Figs. 19-22.—Fixed with sublimate-acetic (95:5) direct. 19, 20. Stained iron-hæmatoxylin; never-dried. 21, 22. Stained Giemsa, then dried off, showing extraordinary shrinkage. Compare figs. 14, 15, etc.

Figs. 23, 24.—Osmic vapour, followed by Schaudinn's fluid; stained iron-hæmatoxylin; never-dried. Note the very distinct periplast-line. Compare figs. 40-44. Preserved at the same time from the same blood.

Figs. 25-28.—Fixed with Schaudinn's fluid direct; stained iron-hæmatoxylin; never-dried. 25, much extracted; 26, 27, less; 28, still less extracted. Compare figs. 29-34, 76-78, preserved at the same time from the same blood.

Figs. 29-32.—Fixed with Mann's picrocorrosive without formol; stained iron-hæmatoxylin; never-dried. 29, 30, less, 31, 32, more extracted. Compare figs. 25-28, etc.

Figs. 33, 34.—Fixed with Mann's picrocorrosive with formol; stained iron-hæmatoxylin; moderately extracted; never-dried. Two trypanosomes in the same field showing the relations of kinetonucleus, blepharoplast, and flagellum very sharply. Compare figs. 25-28, etc.

Fig. 35.—Treated as last. Rather more extracted. Compare figs. 1-8, etc.

Figs. 36-38.—Fixed in Flemming's fluid direct; stained iron-hæmatoxylin; never dried. 36, 37, compare with figs. 10, 11, etc. 38, compare with figs. 17, 18, etc.

Fig. 39.—Fixed with osmic vapour followed by Flemming's fluid; stained iron-hæmatoxylin; never-dried. Compare with figs. 14, 15, etc.

Figs. 40-44.—Fixed in Hermann's fluid direct; 40, stained gentian violet and orange; 41, 42, stained safranin, gentian violet, orange; 43, 44, stained iron-hæmatoxylin; never-dried. Compare figs. 23, 24, etc.

Figs. 45-53.—Nuclei of trypanosomes from the same preparation as figs. 23, 24. 45 shows the most usual condition, occurring in about 90 per cent. of the trypanosomes.

Figs. 54-59.—Nuclei of trypanosomes from a companion preparation to that from which figs. 33, 34 were drawn; treated in the same manner, but the stain rather more extracted. 54 represents the most usual condition.

PLATE 22.

All figures drawn from preparations stained with the Romanowsky stain, either Giemsa's method (G.) or azure-erythrosin (A. E.).

Figs. 60, 61.—Fixed with osmic vapour, then dried off; fixed with absolute (G.). Compare with figs. 14, 15, etc.

Figs. 62, 63.—Osmic vapour; absolute alcohol; never-dried (G.). Compare with figs. 10, 11, etc.

Figs. 64-69.—From a companion slide to the foregoing; more extracted with acetone (see pp. 775-777).

Figs. 70-72.—Trypanosomes showing folds of the deeply-stained periplast simulating fibrils (see p. 797). 70, fixed osmic vapour, then dried off; sublimate acetic (95 : 5), stained (G.); from a companion preparation to that from which fig. 73 is drawn. 71, 72, osmic vapour, absolute alcohol (G.); never-dried. Compare figs. 1-8, etc.

Fig. 73.—Osmic vapour, sublimate-acetic (95 : 5), stained (G.); dried off after the osmic vapour. Compare figs. 14, 15, etc.

Fig. 74.—Trypanosome with three trophonuclei; osmic; absolute alcohol (G.); dried off.

Fig. 75.—Osmic vapour, sublimate-acetic (A. E.); never-dried. Compare figs. 17, 18, etc.

Fig. 76.—Schaudinn's fluid direct (A. E.); never dried. Compare figs. 25-34, etc.

Figs. 77, 78.—Mann's pierocorrosive with formol (A. E.); never-dried. Companion preparation to the last.

Fig. 79.—Treatment similar to the last. Compare figs. 1-8, etc.

Figs. 80, 81.—Osmic vapour, Flemming's fluid (G.); dried off. Compare figs. 14, 15, etc.

PLATE 23.

All figures drawn from preparations stained with Twort's stain; never-dried.

Figs. 82-84.—Fixed in sublimate-acetic (95 : 5).

Fig. 85.—Fixed in Schaudinn's fluid.

Fig. 86.—Fixed in Mann's pierocorrosive without formol.

Fig. 87. —Fixed in Mann's pierocorrosive with formol.

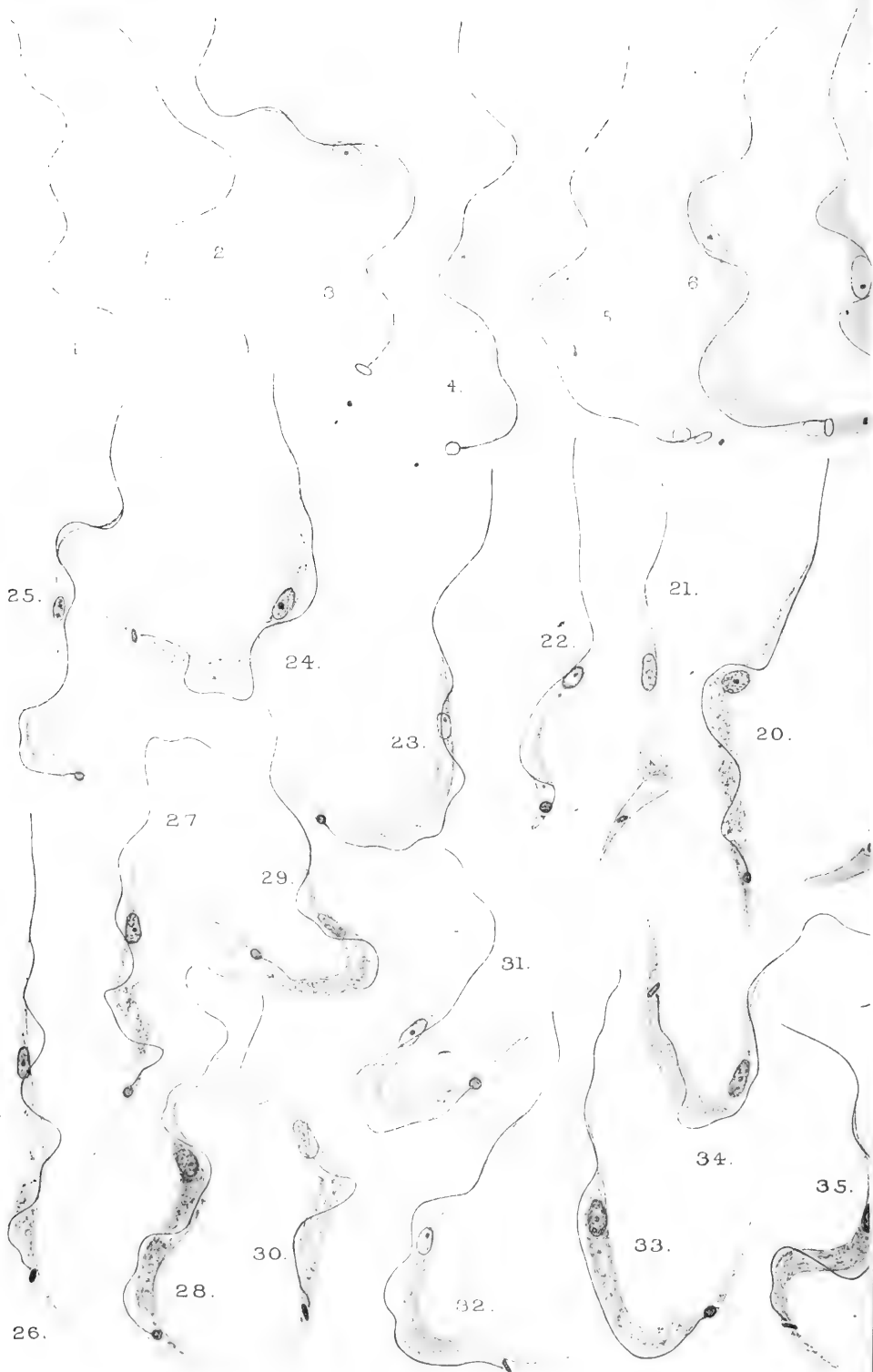
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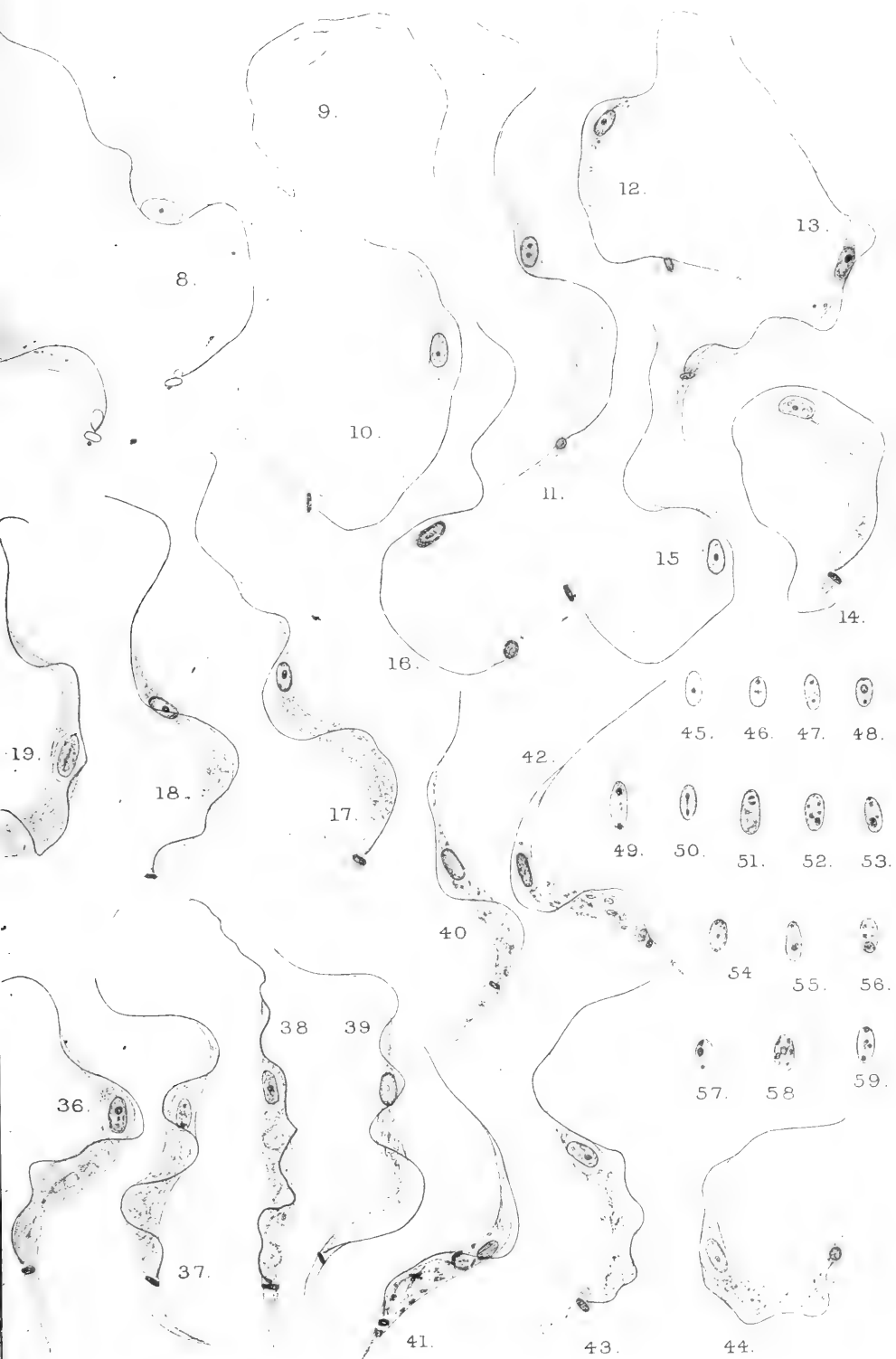
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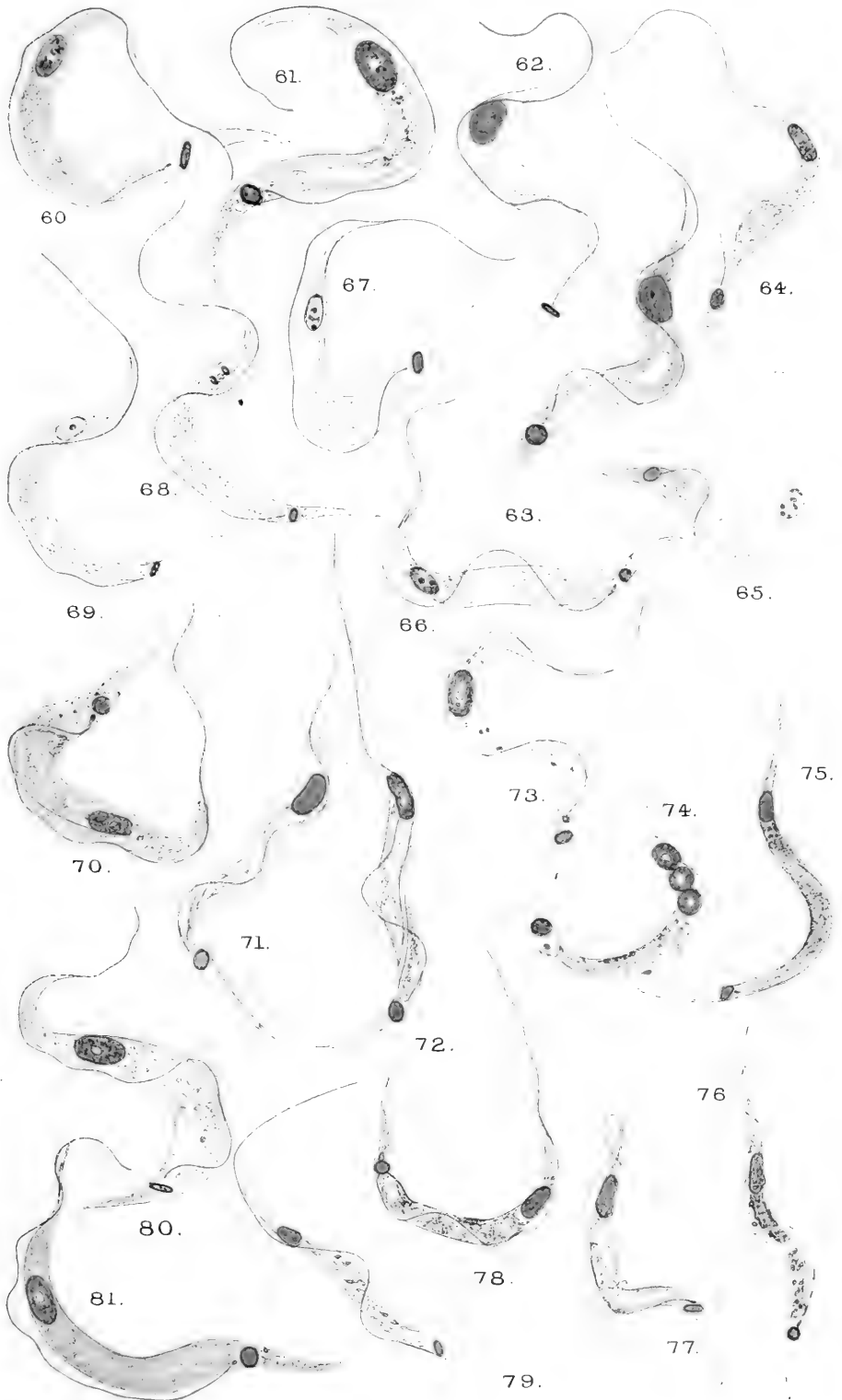
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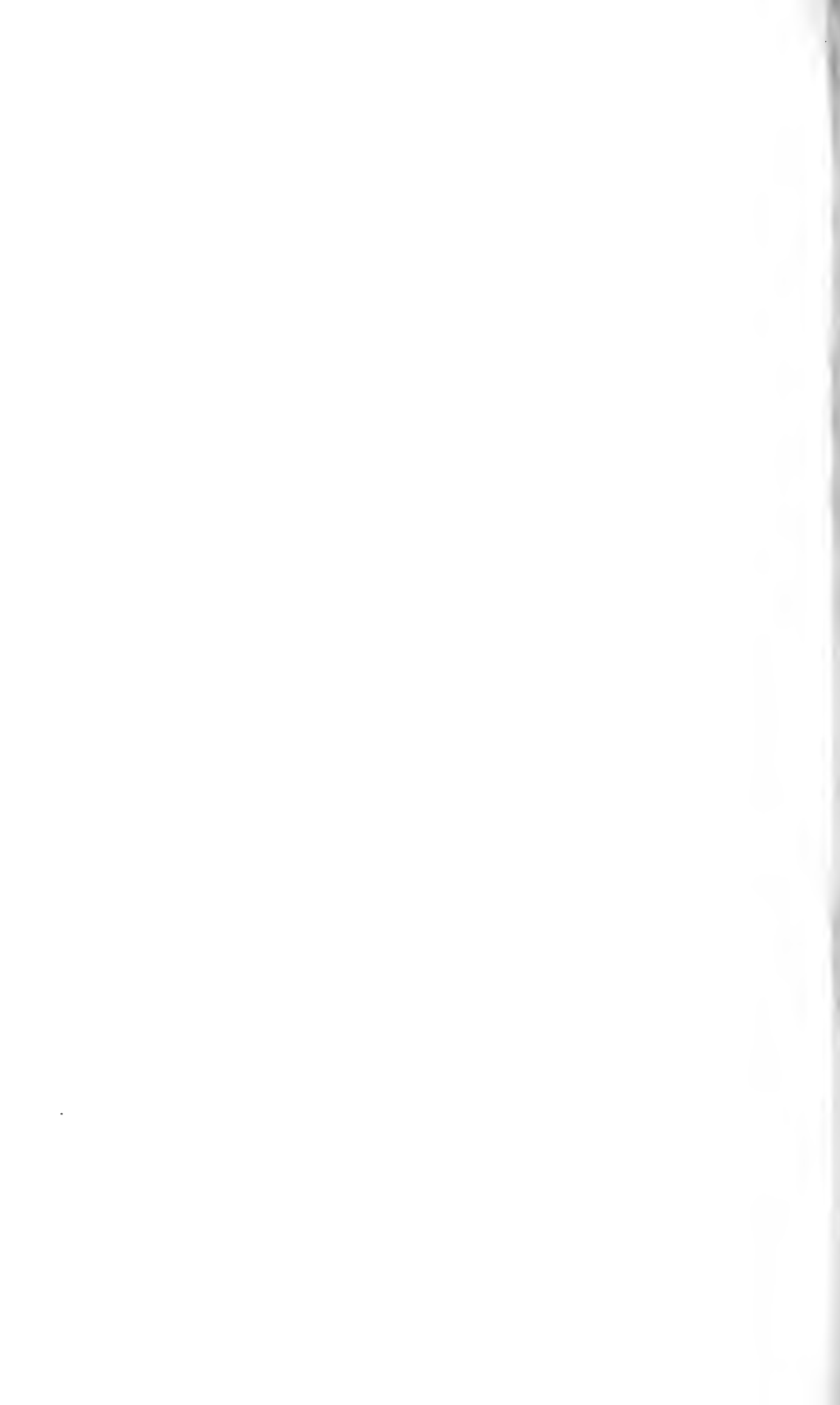


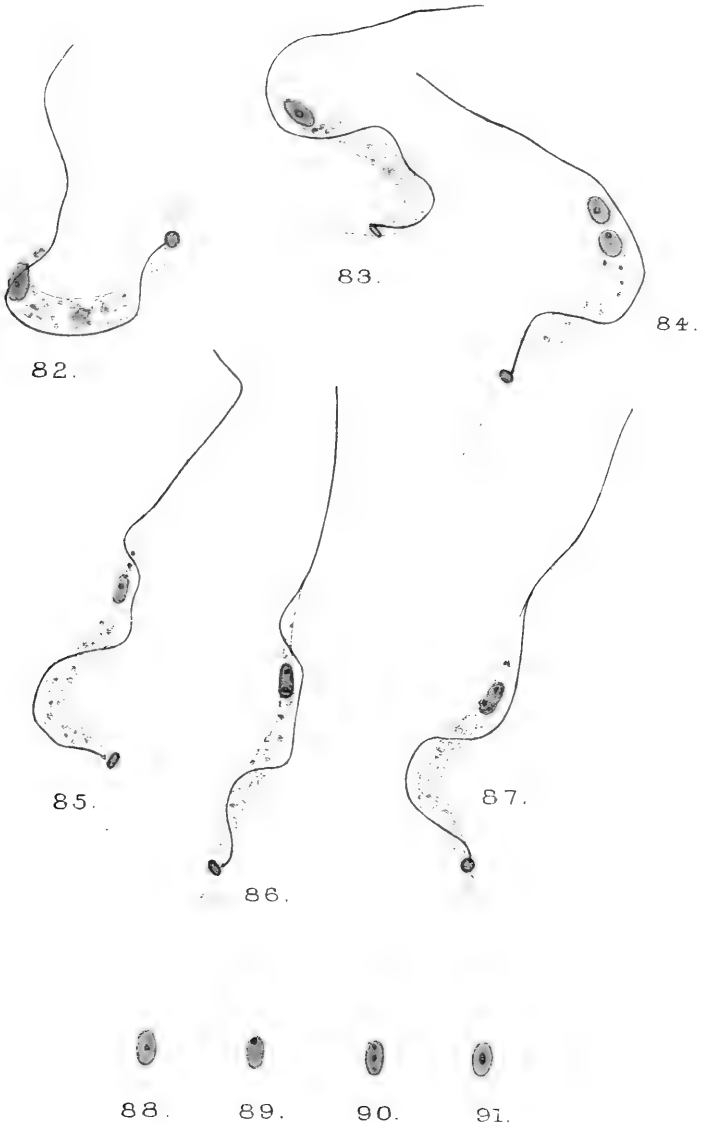


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EDITED BY

SIR RAY LANKESTER, K.C.B., M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD; CORRESPONDENT OF THE INSTITUTE OF FRANCE,
AND OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE ACADEMY
OF SCIENCES OF PHILADELPHIA, AND OF THE ROYAL ACADEMY OF SCIENCES
OF TURIN; FOREIGN MEMBER OF THE ROYAL SOCIETY OF SCIENCES OF
GÖTTINGEN, AND OF THE ROYAL BOHEMIAN SOCIETY OF SCIENCES, AND
OF THE ACADEMY OF THE LINCEI OF ROME, AND OF THE AMERICAN
ACADEMY OF ARTS AND SCIENCES OF BOSTON; ASSOCIATE OF THE
ROYAL ACADEMY OF BELGIUM; HONORARY MEMBER OF THE
NEW YORK ACADEMY OF SCIENCES, AND OF THE
CAMBRIDGE PHILOSOPHICAL SOCIETY, AND OF
THE ROYAL PHYSICAL SOCIETY OF EDIN-
BURGH, AND OF THE

BIOLOGICAL SOCIETY OF PARIS, AND OF THE CALIFORNIA ACADEMY OF SCIENCES OF SAN FRANCISCO, AND
OF THE ROYAL ZOOLOGICAL AND MALACOLOGICAL SOCIETY OF BELGIUM;
CORRESPONDING MEMBER OF THE SENKENBERG ACADEMY OF FRANKFURT-A-M.;
FOREIGN ASSOCIATE OF THE NATIONAL ACADEMY OF SCIENCES, U.S., AND MEMBER OF THE
AMERICAN PHILOSOPHICAL SOCIETY;
HONORARY FELLOW OF THE ROYAL SOCIETY OF EDINBURGH;
LATE DIRECTOR OF THE NATURAL HISTORY DEPARTMENTS OF THE BRITISH MUSEUM; LATE PRESIDENT OF THE
BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE; LATE VULKERIAN PROFESSOR OF
PHYSIOLOGY IN THE ROYAL INSTITUTION OF GREAT BRITAIN;
LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN THE UNIVERSITY OF CAMBRIDGE

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF MANCHESTER;

AND

E. A. MINCHIN, M.A.,

PROFESSOR OF PROTOZOOLOGY IN THE UNIVERSITY OF LONDON.

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By E. RAY LANKESTER, M.A., LL.D., F.R.S.

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